APPENDIX 1-1

Penobscot River Mercury Study

Summary of

Inter-Laboratory Comparisons and Reports

THg and MeHg in Water		**THg and MeHg in Sediments**		**THg and MeHg in Tissues**	
Samples	**Report**	**Samples**	**Report**	**Samples**	**Report**
Sep & Oct 2006	First Report July 2007	May and July 2007	Second Report, Sep 2008		
May and July 2007	Second Report Sep 2008	May & July 2008	Second Report, Sep 2008	Sep 2006 prep Sent May 2007	Second Report Sep, 2008
Sep 2008	Third Inter lab Comparison, Water, March 2009	March 2008	Second Report, Sep 2008	Sep 2006 Prep, Sent June 08	Second Report Sep, 2008
July 2009	Fourth Inter lab Comparison, Water, Dec 2009	August 2009	Sediment Interlab Report, June 2010	Sep 2009	Tissue Interlab Report, August 2010
July 2010	Fifth Inter lab Comparison, Water, October 2011	August 2009, Evers samples, digest vs distillation and extraction	January 2010 Excel sheet 3110, Battelle		

Summary of Quality Control/ Quality Assurance procedures for analyzing Water and Sediment samples—C. Kelly, June 2008

These notes are meant only as a summarizing check list, derived from EPA Methods 1631 for Total Mercury in Water, and 1630 for Methyl Mercury in Water. See these original method descriptions for details.

1. A Chain of Custody record should be kept, recording
    a. time and site of sampling
    b. names of samplers
    c. method of immediate storage (e.g., in cooler, on ice, frozen)
    d. method and date of shipping
    e. condition of samples and date of receipt at laboratory (i.e., if thawed, etc.)
    f. method of storage in laboratory.
2. For water sampling, 3 types of field blanks should be done at a minimum of 3 sites in one sampling trip, or every 5 sites if more than 15 sites are sampled. The 3 types are a trip blank, a bottle blank, and a filtered blank (see details below).
3. Requisite analytical blanks are run each day, as specified in section 9 of each method.
4. Analytical duplicates (repeat analysis on same sample) are run at a frequency of at least 1 in every 20 samples.
5. Currently, field replicates are taken for all water samples collected in the Penobscot system (i.e. 2 samples at each sample site). This might not be appropriate for your sampling scheme. They should be done at some frequency (at least 10%), however, to establish the precision of field sampling.
6. The frequency of field replicates for core or sediment samples should be determined according to the goals of the sediment sampling program. The minimum frequency should be 10%.
7. Matrix spike recoveries are done on each day of analysis.
8. A certified reference material, or quality control sample, is analyzed on each day of analysis (e.g., IAEA 405 for THg and MeHg in sediment, NIST 1641d for THg in water).
9. An "ongoing precision and recovery" standard, made up in each lab, is also analyzed on each day of analysis.
10. All of the above, in addition to detection limits, are reported with each batch of samples that are analyzed. A "batch" is 20 samples, or less than 20 if fewer than 20 are done in one day.
11. The acceptable limits for matrix spike recovery efficiencies, reference material recoveries, OPR results, blanks, and detection limits are as set out in the EPA method descriptions.
12. Each laboratory will participate once or twice a year in an interlab comparison exercise, more frequently if problems are detected.

Field Blank procedure used in collection of Penobscot water samples:

1. The laboratory will provide Milli-Q water (that has been analyzed for THg) for the field crew to use in all the field blank procedures. One 2-liter bottle will be provided for each site where a set of blanks will be done. Three clean sample bottles (500 mL each) will also be provided for each site.
2. Blanks will be done at a frequency appropriate to the sampling program. Blanks should be prepared when the field crew first arrives at the site and takes out the sampling equipment, and before taking samples.
3. All blanks will be prepared in the field using the same clean hands/dirty hands techniques used in collecting water samples.
4. At each site where field blanks are done, 3 types of blanks will be prepared:
    a. **Bottle Blank**—Milli-Q water will be poured directly from the lab-supplied bottle into a water sampling bottle. This checks the sampling handling technique, and possible contamination of sample bottles.
    b. **Unfiltered Blank**—Milli-Q water will be pumped from the lab-supplied bottle into a water sampling bottle, using the same pump and sampling line used in water sampling, but with no in-line filter. A small amount of Milli-Q water should be used to rinse the line before filling the sample bottle, as this rinsing is the normal procedure when taking a sample. This checks for any contamination of the pump and sample line.
    c. **Filter Blank**—Milli-Q water will be pumped through the in-line filter into a water sampling bottle. A small amount of Milli-Q water should be used to rinse the filter before filling the sample bottle. This checks for any contamination of the filter.
5. All blanks will be kept in the same large containers as the field sample bottles during storage and transport to the analytical lab.
6. Any unused Milli-Q water should be returned to the analytical lab in its original bottle.

Summary of Field Replicate Results
for Penobscot Water Samples,
2006 and 2007

When sampling water from the Penobscot river and estuary, in the 5 sampling periods from late August, 2006 through July 2007, replicate samples of water were taken in the field. This means that two separate bottles were taken at each site, for each of the four mercury analyses that would be done later (filtered methyl mercury, unfiltered methyl mercury, filtered total mercury, and unfiltered total mercury). In addition, for the last 3 sampling periods (October 2006, May 2007 and July 2007), replicate samples were taken for total suspended solids (TSS).

The purpose of the replicate sampling program was to determine the reproducibility of the combined sampling and analytical efforts. This is always an issue in waters where currents may cause spatial heterogeneity in surface water concentrations. It is also important when measuring trace level substances such as mercury, where contamination of samples during handling is always a concern, and where analyses in the laboratory must contend with extremely low levels of the analyte. Good replication is an indication of good sample handling, but it should also be kept in mind that there is natural heterogeneity in water systems, especially for particulates. Thus, it is expected that the reproducibility for filtered waters will be better than for unfiltered waters, which includes the samples for TSS.

In each sampling period, 28 to 38 replicates (pairs) of samples were taken for each analysis. The relative percent difference (RPD = difference between the two replicates divided by the average of the two replicates) was calculated for each pair.

Replication for both filtered and unfiltered total mercury was very good, averaging from 3 to 13% (Table 1).

Table 1. Relative percent Difference (RPD) for replicate water samples analyzed for filtered total mercury and replicate samples analyzed for unfiltered total mercury.

	THg filtered ng/L			**THg unfiltered ng/L**		
	Average RPD	Std Dev	n	Average RPD	Std Dev	n
Period II	11.4%	11.1%	33	6.4%	7.5%	32
Period III	10.5%	13.0%	37	5.0%	5.5%	37
Period IV	9.6%	8.1%	35	10.3%	9.0%	34
Period V	6.7%	8.6%	35	5.9%	8.1%	35
Period VI	4.1%	3.0%	35	11.4%	14.9%	35

The variability for replicate water samples taken for filtered and unfiltered methyl mercury was greater than for total mercury (Table 2). This is somewhat expected because the methyl mercury concentrations are much lower than total mercury concentrations, and more difficult to measure analytically.

Table 2. Relative percent Difference (RPD) for replicate water samples analyzed for filtered methyl mercury and replicate samples analyzed for unfiltered methyl mercury.

	MeHg filtered ng/L			**MeHg unfiltered ng/L**		
	Average RPD	Std Dev	n	Average RPD	Std Dev	n
Period II	19.2%	30.5%	38	28.1%	23.8%	34
Period III	23.0%	22.5%	36	25.8%	24.8%	37
Period IV	21.8%	15.2%	28	33.4%	23.8%	29
Period V	15.6%	15.8%	35	12.2%	8.2%	35
Period VI	18.5%	21.5%	35	15.9%	15.0%	35

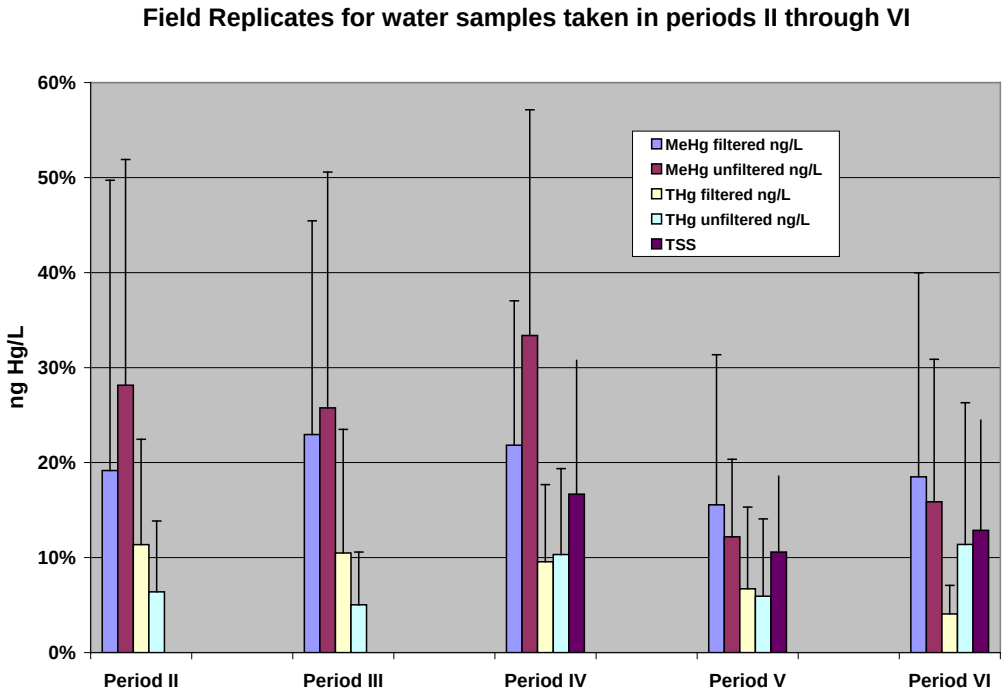
Samples were taken for total suspended solids only in sampling periods IV, V and VI. The relative percent difference was fairly low, averaging 10.6 to 16.7% in the different sampling periods (Table 3). Variability in TSS was slightly higher than for the total mercury samples, and not as high as for the methyl mercury samples.

Table 3. Relative percent Difference (RPD) for replicate water samples analyzed for total suspended solids.

	Total Suspended Solids		
	Average RPD	Std Dev	n
Period IV	16.7%	14.1%	34
Period V	10.6%	8.0%	35
Period VI	12.9%	11.6%	35

A summary of both the average relative per cent difference, and the standard deviations on these averages, of all replicate data are shown in Figure 1. The standard deviations show that there was a larger degree of variability in the RPD's for sampling pairs for both filtered and unfiltered methyl mercury than for total mercury and TSS.

Also, for methyl mercury, reproducibility was slightly better in the last two sampling periods. For total mercury, reproducibility in sampling and analyses was consistent throughout all periods. Reproducibility for TSS was also consistently good, considering that particulates can be quite variable in water samples. Taken all together, the data indicate that sample handling and analyses may have improved slightly in 2007. Certainly there is no indication that these have worsened.



Report on Interlab Comparison and Quality Control/Quality Assurance Data from Laboratories participating in the Penobscot Mercury Study

C.A. Kelly
R&K Research Inc.
July 9, 2007

Contents

I. Summary of recommendations

1. The QA/QC data need to be examined in a timely manner, in order to detect and to correct problems immediately.

2. Repeat the interlab comparison for methyl mercury in sediments, with all laboratories doing both extraction and distillation as the initial preparatory step.

3. The field blank results should be noted by both the laboratory and the field personnel. Total mercury concentrations in field blanks for sampling periods II and III in 2006 were higher than desirable, indicating contamination in one or more of a) bottles provided for sampling by Studio Geochimica, b) water provided by Studio Geochimica, or c) field techniques. The field blank data should be kept in mind when analyzing data for THg in water for periods II and III, compared to period IV.

4. The Battelle Laboratory should check the COC (chain of custody) sheets for samples transferred from Studio Geochimica in 2006 by comparing the Normandeau field notes (bottle #, site description, date) to the final sample descriptions on the data sheets.

II. Descriptions of Methods and Quality Assurance/Quality Control Procedures of the Laboratories participating in the Penobscot Mercury Study

Flett Research

Total Mercury in water:

Analytical Method: EPA 1631e, Total Mercury in Water by Oxidation, Purge and Trap, and CVAFS (FR internal method #T00120, version 3).

Detection Limit: Minimum detection limit **(**MDL) = 0.04 ng Hg/L (based on 7 replicates of analytical blanks (99% confidence level)). The practical method limit (ML) of 0.5 ng/L, as stated in Method 1631e, has been adopted for our laboratory to reflect occasional elevated bottle blanks (< 0.5 ng/L) observed in reused acid-cleaned Teflon bottles filled with DI water.

Estimated uncertainty: The estimated uncertainty of this method has preliminarily been determined to be ± 14.7 % @ 95 % confidence at a concentration level of 0.2 - 50 ng/L.

Quality assurance material: OPR (Ongoing Precision Reference) solutions, which are large batches of water made up with a THg concentration within the range of usual samples. Each batch is large enough to provide a reference sample that is run on multiple consecutive dates, to check for day to day variance in analytical results. A second material is Baker Quality Control Solution (QCS), with a certified concentration of 1000 ng/L.

Methyl mercury in water

Analytical Method: EPA (proposed) Method 1630; FR internal method # M10110 (Version 3): Methyl Mercury in water by distillation, ethylation, purge and trap, and CVAFS.

Detection Limit: MDL = 0.048 ng/L; ML = 0.14 ng/L. The method detection limit (MDL) is calculated to be the concentration equivalent to approximately three times the standard deviation of replicate measurements of the analyte in the given matrix at a concentration at or near the detection limit. (99% confidence level, 6 degrees of freedom). Client results are flagged below the ML.

Estimated Uncertainty: The estimated uncertainty of this method has preliminarily been determined to be ± 22 % at 0.5 ng/L (95 % confidence)

Quality assurance material: On each day when analyses are done, a certified reference material (MeOPR, 1000ng/L) is analyzed and compared to the certified concentration (the expected concentration).

Total Mercury in sediment

Analytical Method: Total Mercury in Sediment, Soil, and Peat by Digestion, Purge and Trap, and CVAFS, adaptation of EPA Method 1631e, FR internal method # T00130, version 3.

Detection Limit: e.g. MDL = 2.4ng/g The method detection limit (MDL) is calculated to be the concentration equivalent to approximately three times the standard deviation of replicate measurements of the analyte in method blanks. (99% confidence level, 6

degrees of freedom) This limit assumes a 100 mg sample size. Lower detection limits are possible if greater sample weights are used.
Estimated Uncertainty: Preliminarily determination: ± 18 % @ 95 % confidence at a concentration level of 40-100 ng/g; ± 32 % @ 95 % confidence at a concentration level of < 15 ng/g.
Reference Material: On each day when analyses are done, a certified reference material (Mess-2, 92ng Hg/g, from the National Research Council) is analyzed and compared to the certified concentration (the expected concentration).

Methyl mercury in sediment
Analytical Method: Methyl Mercury in sediment by distillation, ethylation, purge and trap, and CVAFS, adaptation of EPA Method 1630, FR internal method #M10140, Version 3.
Detection Limit: MDL = 0.02 ng/g based on 7 replicates of analytical blanks (99% confidence level).
Estimated Uncertainty: ± 18.3 % @ 95 % confidence at a concentration level of 0.1-50 ng/g
Reference material: On each day when analyses are done, a certified reference material (IAEA 405, 5.49 ng MeHg/g ±0.53, from the International Atomic Energy Agency) is analyzed and compared to the certified concentration (the expected concentration).

Matrix effects on recovery of Methyl Mercury or Total Mercury
On each day when analyses are done, the water, sediment or tissue are spiked with a known amount of total mercury or methyl mercury. The analytical values obtained after spiking is compared to unspiked values, and then the recovery efficiency of the spike is calculated. This takes into account the effects of the different materials (matrices), in which the mercury or methyl mercury is found, on the analytical method being used.

Battelle Sequim Laboratory

Total Mercury in Water: All samples stipulated for both total mercury and methylmercury were distilled by the method of Horvat, et al., 1993 for methyl mercury. Samples for total mercury were analyzed by EPA Method 1631e. Both unfiltered and filtered water samples were digested for total mercury by subjecting them to bromine monochloride oxidation for a minimum 24 hours. Mercuric ions in the oxidized sample were reduced to Hg° with $SnCl_2$, and then purged onto gold-coated sand traps as a means of preconcentration and interference removal. Mercury vapor was thermally desorbed to a second "analytical" gold trap, and from that into a fluorescence cell. Fluorescence (peak area) is proportional to the quantity of mercury collected, which is quantified using an average response factor as a function of the quantity of sample purged. On receipt of samples, the internal temperature of the cooler used for transport is measured, in order to record if any coolers are received at temperatures above the recommended temperature of $\leq 8^{o}$ C. Samples are analyzed within the EPA holding time of 90 days.

Methyl Mercury in Water: All samples stipulated for both total mercury and methylmercury were distilled by the method of Horvat, et al., 1993 for methyl mercury. Samples were analyzed for methyl mercury by EPA Method 1630. Methylmercury in the distilled sample was ethylated and then purged onto carbon traps as a means of preconcentration and interference removal. The ethylated methylmercury was thermally desorbed into a fluorescence cell. Fluorescence (peak area) is proportional to the quantity of methylmercury collected, which is quantified using an average response factor as a function of the quantity of sample purged. Samples were analyzed within the EPA holding time of 180 days.

Total Mercury in Sediment: All samples stipulated for total mercury were analyzed for total mercury by EPA Method 7473 (Thermal Decomposition, Amalgamation, and Cold Vapor Atomic Spectrophotometry). Samples were analyzed within the EPA holding time of 180 days. The standard reference material was IAEA-405. The criteria for recovery was 80-120%.

Methyl Mercury in Sediment: All samples stipulated for methylmercury were extracted by the method of Bloom et al, 1997 for methyl mercury. Samples were analyzed by a modification of EPA Method 1630. Methylmercury in the extracted sample was ethylated and then purged onto carbon traps as a means of preconcentration and interference removal. The ethylated methylmercury was thermally desorbed into a fluorescence cell. Fluorescence (peak area) is proportional to the quantity of methylmercury collected, which is quantified using an average response factor as a function of the quantity of sample purged. Samples were analyzed within the EPA holding time of 180 days. The standard reference material was IAEA-405. The criteria for recovery was 65-135%.

Table 1. Methods used by the three laboratories participating in the interlab comparison. Flett Research and Battelle use Cold Vapor Atomic Flourescence Spectroscopy (CVAFS) for mercury detection, while Trent U. uses ICP/MS with isotopic dilution.

	THg in Water	MeHg in Water	THg in sediments	MeHg in sediments
Flett Research	EPA Method 1631e	EPA Method 1630	EPA Method 1631e	EPA Method 1630 with distillation
Battelle	EPA Method 1631e	EPA Method 1630	EPA Method 7473	EPA Method 1630 with extraction
Trent U.	EPA Method 1631e	EPA Method 1630	EPA Method 1631e	EPA Method 1630 with distillation

III. Field Blanks

Deionized (DI) or Milli-Q water was sent in a large bottle to the field, and was poured into Teflon sample bottles for "unfiltered" field blanks, and was filtered through the in-line filter for "filtered" field blanks.

Field blank values were above the detection limits for both THg (0.121ng/L) and MeHg (0.0192 ng/L). In most cases, filtered values were not significantly higher than unfiltered values, indicating that the filtration apparatus did not result in contamination of the water being filtered. There were three occasions, however, when filtered THg values were more than 50% higher than unfiltered values, indicating a possible problem in rinsing the filtration line after the previous sample.

Table 2. Results for field blanks carried out during each sampling period in 2006. Analyses were done at Battelle Sequim Laboratory.

Sampling Period		THg unfiltered ng/L	THg filtered ng/L	MeHg unfiltered ng/L	MeHg filtered ng/L
II	range	0.39-.55	0.40-0.83	n.d. to 0.19	n.d. to 0.14
II	**mean**	**0.479**	**0.528**	**0.040**	**0.033**
II	s.d.	0.054	0.144	0.073	0.050
	n	7	7	6	6
		0	0	0	0
III	range	0.21-0.56	0.22-0.72	n.d. to 0.04	n.d. to 0.032
III	**mean**	**0.439**	**0.452**	**0.022**	**0.024**
III	s.d.	0.123	0.150	0.014	0.008
	n	7	7	7	7
		0	0	0	0
IV	range	n.d. to 0.16	n.d. to 0.44	n.d. to 0.024	n.d. to 0.023
IV	mean	**0.143**	**0.235**	**0.017**	**0.015**
IV	s.d.	0.018	0.105	0.006	0.006
	n	6	8	6	8

The field blank results for THg in sampling periods II and III averaged 0.47 to 0.45 ng/L, which was higher than what is considered desirable for low level mercury sampling (0.1 to 0.3 ng/L). The water used for sampling periods II and III was sent from Studio

Geochimica and was not analyzed prior to being sent out to the field, whereas the water used for sampling period IV (when field blank results were within the acceptable range) was analyzed and was below detection. Also, water samples sent to SG during these two sampling periods were transferred from the original sample bottles to glass bottles before being sent on to Battelle. Thus, problems other than field handling cannot be ruled out for periods II and III. Most unfiltered field blanks in these two periods were 0.4 to 0.5 ng/L, showing a certain consistency. Filtered field blanks showed less consistency, ranging from 0.2 to 0.8 ng/L. The results for all water samples should be examined to see if there is a way to determine whether high field blanks meant that samples could have had higher than expected results in samples where THg concentrations were low.

Field blank values for total mercury in water were lower in Sampling Period IV than in the two earlier periods, but the exact reasons for this are still unclear. These field blank values should be watched closely during the next sampling periods, for both filtered and unfiltered water samples.

Table 3. Results of analyses of field blanks for all sampling periods in 2006. Data are from Battelle Sequim Laboratory.

Sampling Periods		THg unfiltered ng/L	THg filtered ng/L	MeHg unfiltered ng/L	MeHg filtered ng/L
II-IV	range	0.010 to 0.586	0.169 to 0.878	n.d. to 0.0585	n.d. to 0.140
II-IV	**mean**	**0.364**	**0.397**	**0.026**	**0.023**
II-IV	s.d.	0.168	0.181	0.041	0.027
	n	20	22	19	21

Overall, there were a number of questions regarding the field blank data that could not be resolved from the 2006 data. Thus, special care should be taken in the field as water sampling continues. Field blank results are a crucial part of quality assurance procedures, and results should always be examined in a timely manner after each sampling period.

IV. Field Replicates

Field replicates are samples taken at the same time and place in the field, but in separate bottles or containers, and analyzed separately in the laboratory. It is expected that the relative percent differences (RPD's) between pairs of field replicates will be greater than the RPD's between pairs of analytical duplicates, which are taken from the same field sample. The degree to which the RPD's are greater is an indicator of 1) natural variability derived from the fact that no two field samples will be exactly alike since they are taken at different points in time and/or from slightly different spots, and 2) variability

that results from handling of samples by the field crew, e.g., if the tube used to collect water samples were “dirty”, then the first replicate might have a higher Hg content than the second replicate, because sample water flowing through the tube would clean the tube before the second sample was taken. Field replicates were taken for total and methyl mercury in filtered and unfiltered water samples, and for core samples.

The RPD’s for THg in unfiltered water (Table 4) were surprisingly low, given the possibility that different numbers of particulates in different water samples can often contribute to variation in THg concentrations. There was one set of samples (EB5 surface, taken Sep 6/06) that showed considerable differences among replicates. However, this was the only set. The average RPD for unfiltered THg field replicates (4.5%) was about the same as the average RPD for analytical duplicates for filtered THg (4.1%, see Table 7). The average RPD for filtered THg samples was somewhat higher (10.4%), but probably not cause for concern. The average RPD for field replicates of filtered MeHg samples (11.9%), was also not much higher than the average RPD for analytical duplicates (7.4%, Table 7). The highest values for average RPD in field replicates, compared to analytical duplicates, was for unfiltered MeHg samples (26.2%). This was likely due to the low concentrations in these samples (most less than 0.15 ng/L), which means that even a few particles containing MeHg could provide a significant difference in two different field samples.

Table 4. Relative percent differences (RPD’s) between pairs of field-replicated water samples. Data from Battelle Sequim Laboratory.

Field Replicates	THg unfiltered	THg filtered	MeHg unfiltered	MeHg filtered
Average RPD	4.5%	10.4%	26.2%	11.9%
STD	4.6%	9.3%	23.0%	10.3%
n	23	23	22	22

Figure 1. Field Replicates THg unfiltered water

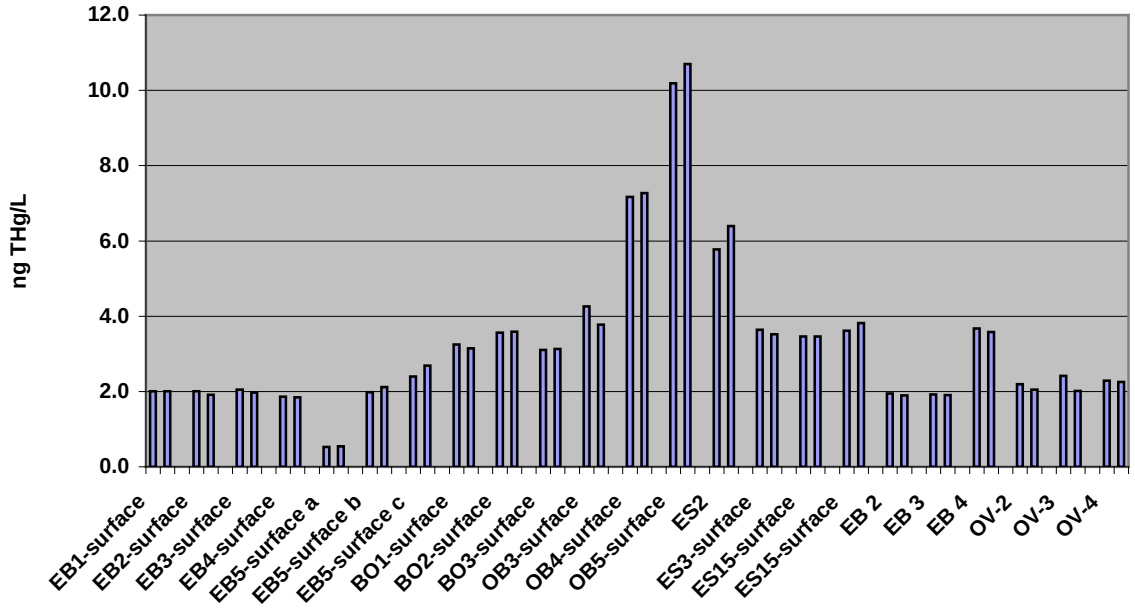


Figure 2. Field Replicates THg filtered water

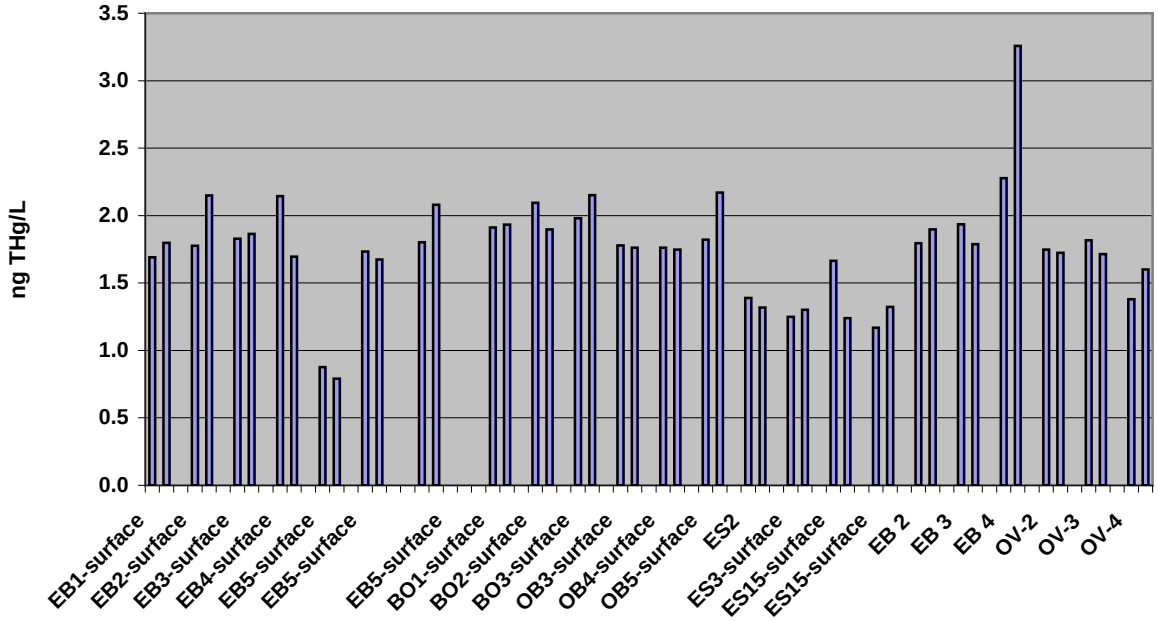


Figure 3. Field Replicates MeHg unfiltered water

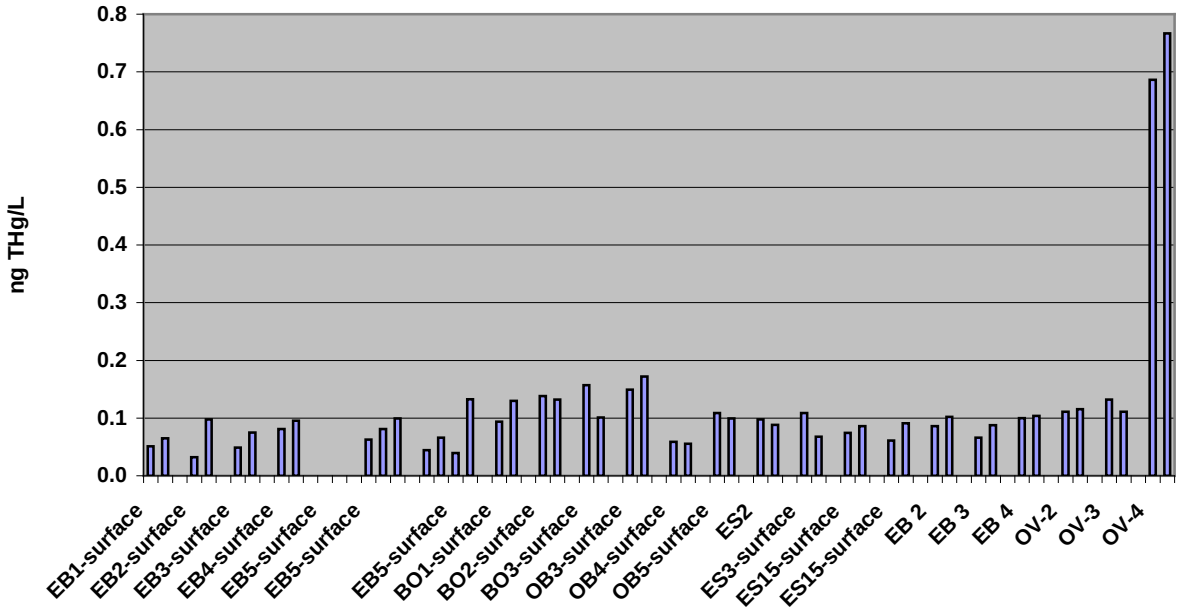
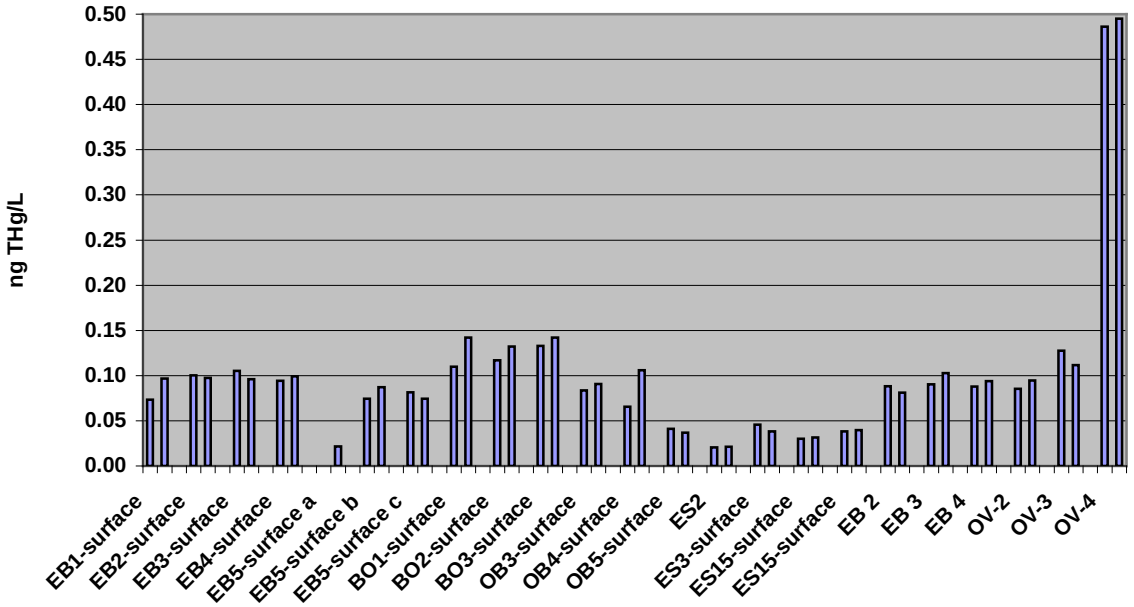


Figure 4. Field Replicates MeHg filtered water



Field replicates for THg in sediment had an average RPD of 10-15% (Tables 5 and 6), compared to an average RPD of 6.5% for analytical duplicates (Tables 8 and 9). This seems acceptable, because sediment core samples are inherently more variable than water samples, and this is seen in the fairly large standard deviations on the means (Tables 5 and 6). Field replicates for MeHg in sediment had an average RPD of 11-16% (Tables 5 and 6) compared to 8.6% for analytical duplicates. Again, this seems very acceptable, in that the results do not indicate any obvious problem with field sampling of sediments.

Table 5. Field replicates, sediment samples. Data from Battelle Sequim Laboratory.

	THg	MeHg
Average RPD	15.21%	11.10%
STD	18.31%	10.03%
n	13	16

Table 6. Field replicates, sediment samples. Data Flett Research Ltd.

	THg	MeHg
Average RPD	10.06%	16.05%
STD	8.25%	20.03%
n =	8	6

V. Analytical duplicates

As part of ongoing precision monitoring, laboratories analyze duplicate subsamples, in order to detect variability in the operation of the mercury analytical procedures. Analytical duplicates are measurements made on one field sample, separated into two duplicate aliquots in the lab, and analyzed separately. The relative percent difference between the two duplicates is expressed as

$$RPD = ((S1-S2)/(S1+S2)/2))*100$$

The RPD's for analytical duplicates for all water and sediment analyses were low (Tables 7, 8, and 9), indicating good precision in the laboratories carrying out these analyses.

Table 7. Relative percent differences between pairs of duplicate water samples. Data are composited from both Flett Research Ltd. and Battelle Sequim Laboratory.

	THg filtered	MeHg filtered
Average RPD	4.13%	7.37%
STD	7.55%	9.55%
n =	17	10

Table 8. Relative percent differences between pairs of duplicate sediment samples. Data are from Battelle Sequim Laboratory.

	THg	MeHg
Mean	6.52%	8.66%
STD	5.63%	7.43%
n	13	9

Table 9. Relative percent differences between pairs of duplicate sediment samples. Data are from Flett Research Ltd.

	THg ng/gdw	MeHg ng/gdw
Average RPD	9.83%	9.26%
STD	5.51%	7.73%
n =	4	10

VI. Interlab Comparison

Samples of both water and sediment were split into three aliquots, and sent to three laboratories for analyses.

All water samples were filtered, because including particulates in split samples can lead to variation that is unrelated to each laboratory's analytical capability. Analyses of THg and MeHg in filtered water showed good agreement among laboratories, with better agreement at higher than at lower concentrations, as would be expected (Figure 5 and 6).

Total mercury in sediments showed very good agreement among the three labs (Figure 7). Methyl mercury results, however, were markedly different, with results from Battelle Sequim Laboratories about a factor of 2 lower than results from Flett Research and Trent University (Figure 8). Battelle used a different method for MeHg, which begins with extraction rather than distillation, and has been shown to result in more accurate results in some sediments that have high total Hg. This is because a small portion of the total Hg can be converted to MeHg during the distillation step. For example, in the OB2

sediments, if 1-2 % of total Hg (which was 500-600 ng/g, Figure 7) were converted to MeHg during distillation, this would account for the 5-10 ng/g higher results for MeHg (Figure 8) in the labs doing this step, compared to the lab doing extraction. The two different methods (extraction and distillation) should be done in each laboratory in order to sort out whether this is the explanation for the differences among the laboratories.

Figure 5. Interlab Comparison, THg, Filtered Water

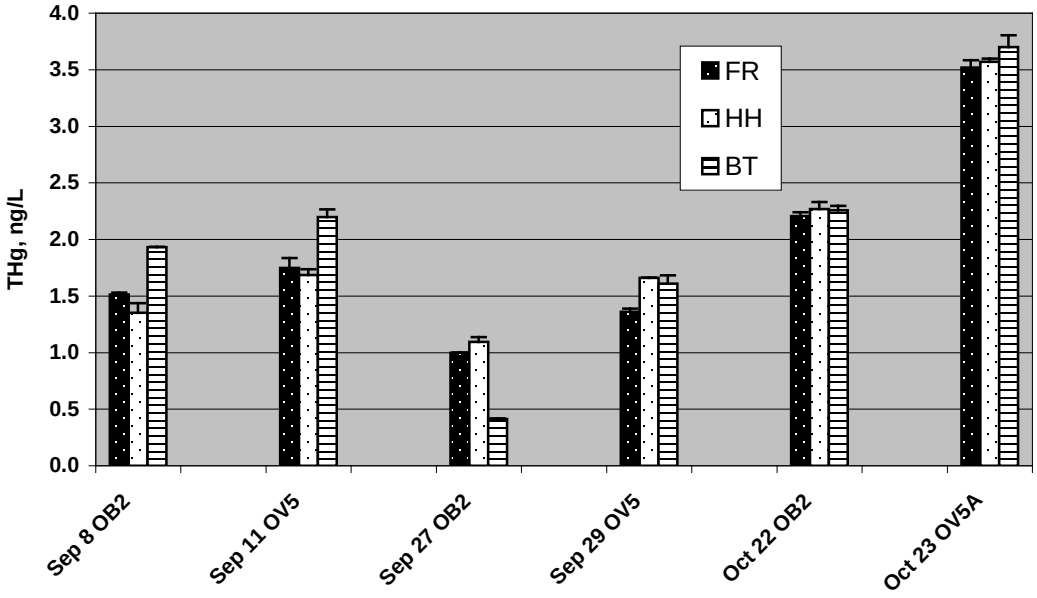


Figure 6. Interlab Comparison, MeHg in Filtered Water

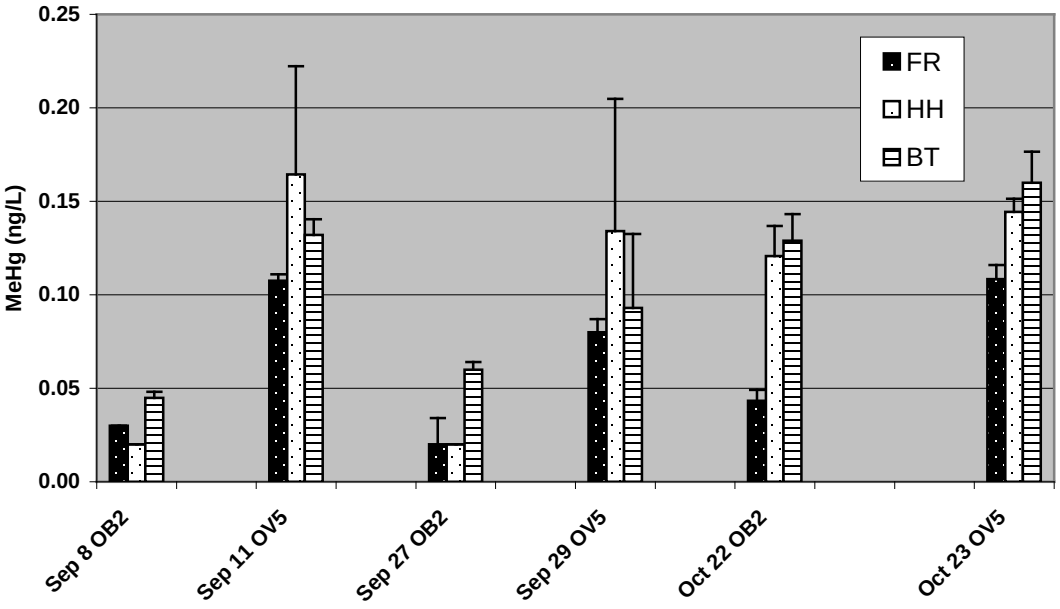


Figure 7. THg in Sediments, Interlab Comparison

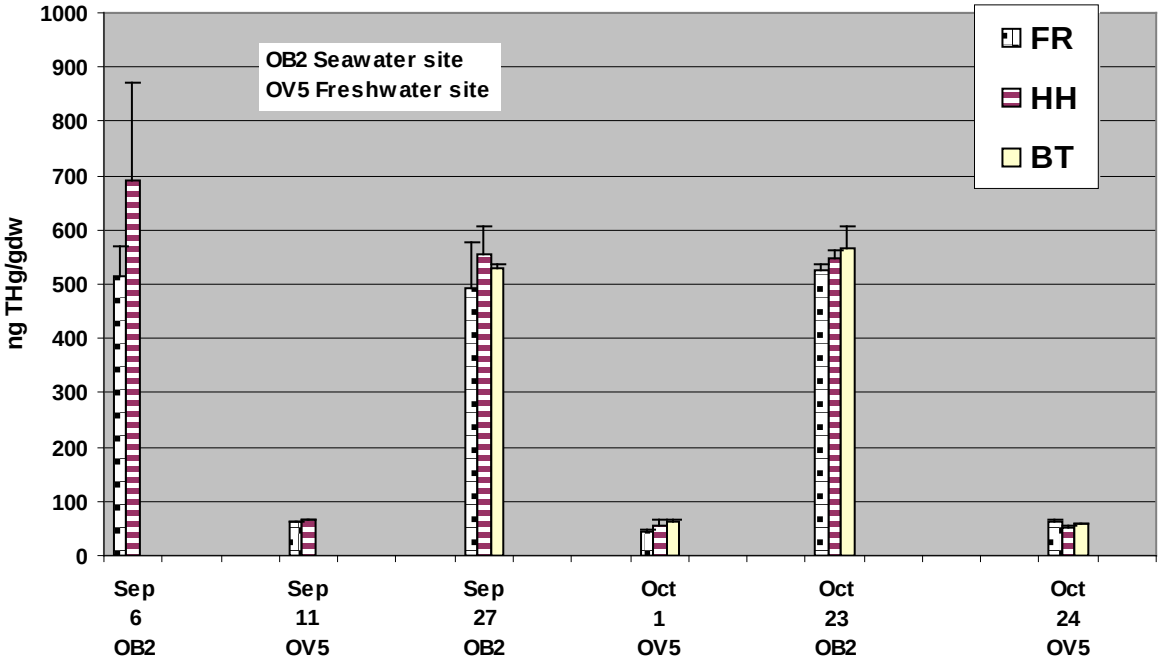
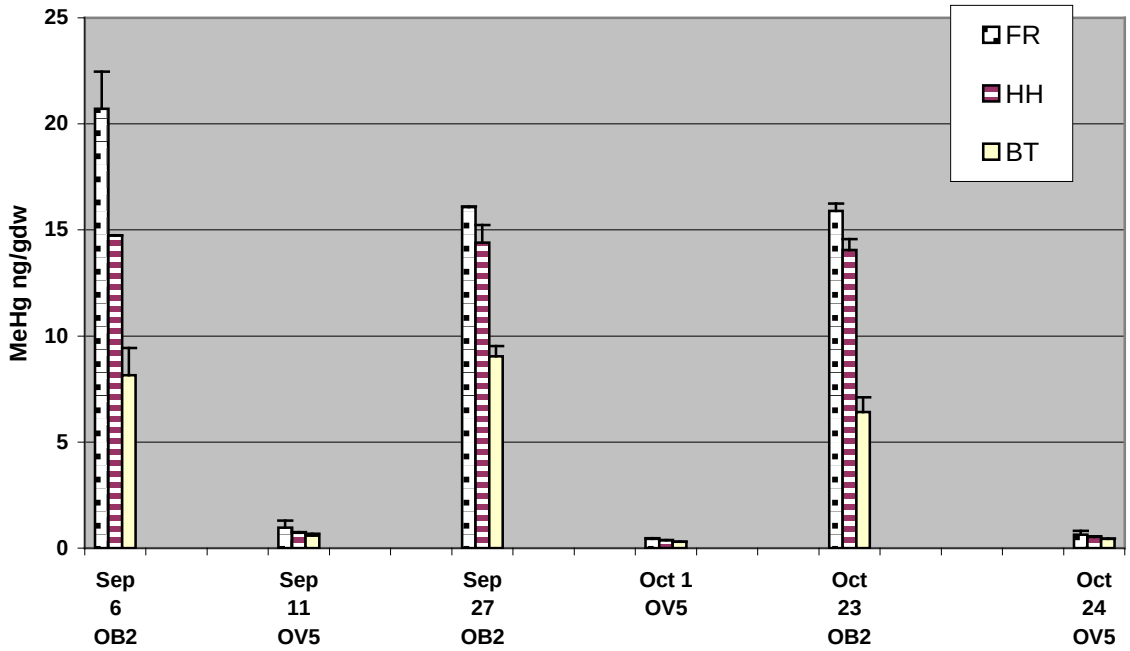


Figure 8. MeHg in Sediments, Interlab Comparison



Appendix A. Raw data for field blanks

station	date	sampling period	unfiltered bottle #	filtered bottle #	THg unfilt ng/L	THg filtered ng/L	% increase after filtering	MeHg unfil ng/L	MeHg filtered ng/L
ES5	9 8 06	II	PC082	PC096	0.507	0.566	11.6%	0.010	0.019
ES10	9 11 06	II	PC087	PC015	0.392	0.480	22.5%		
ES15	9 8 06	II	SGD074	SGD061	0.444	0.495	11.6%	0.010	0.010
BO5	9 10 06	II	PC056	PC018	0.471	0.405	-14.0%	0.190	0.136
EB5	9 6 06	II	PC054	PC034	0.536	0.834	55.7%	0.010	0.016
OV5	9 11 06	II	189447	189446	0.546	0.466	-14.7%	0.010	0.010
OB5	9 8 06	II	PC023	PC051	0.460	0.449	-2.5%	0.010	0.010
EB5	9 26 06	III	PD16	PD037	0.560	0.546	-2.6%	0.023	0.018
OV5	9 29 06	III	PC078	PC074	0.211	0.226	6.9%	0.010	0.025
OB5	9 26 06	III	PC064	PC086	0.450	0.388	-13.8%	0.010	0.020
BO5	9 28 06	III	PC021	PC063	0.486	0.430	-11.5%	0.041	0.032
ES5	9 26 06	III	PC015	PC104	0.439	0.717	63.4%	0.010	0.010
ES10	9 28 06	III	PC045	PC095	0.361	0.438	21.4%	0.022	0.028
ES15	9 28 06	III	SGD088	PC019	0.565	0.420	-25.6%	0.042	0.033
BO3	10 22 06	IV	185665	185666	0.141	0.305	116.3%	0.010	0.010
ES12	10 24 06	IV	185775	185776	0.160	0.226	41.3%	0.010	0.023
ES6	10 23 06	IV	na	Field Blank		0.289			0.010
ES6	10 23 06	IV	na	Equip Blank		0.438			0.010
ES5	10 24 06	IV	185773	185774	0.157	0.169	7.6%	0.024	0.010
BO5	10 22 06	IV	Unfilt. Blk	Filt. Blk	0.121	0.139	14.9%	0.019	0.019
OV4	39013	IV	Unfilt. Blk	Filt. Blk	0.121	0.121	0.0%	0.019	0.019
EB-1	39014	IV	185771	185772	0.158	0.191	20.9%	0.019	0.019

Summary of Field Blanks, Summer 2007

C.A. Kelly
Mercury analyses -- Battelle Marine Sciences Laboratory
Blank water --Battelle
Field procedures—Normandeau Assoc.

May 31-June 2/07	MeHg ng/L		THg ng/L	
Blank Water Sent to Field 4/23/07	0.0188	U	0.188	U
Average, unfiltered blanks, n = 5	0.019		0.213	
Average, filtered blanks, n = 5	0.018		0.192	
Achieved Detection Limit	0.0188		0.188	
Samples				
Unfiltered, mean +/- S.D.	0.143 +/- 0.062		2.95 +/- 0.90	
Filtered, mean +/- S.D	0.112 +/- 0.056		1.945 +/- 0.631	
Low samples, filtered, range	0.02 to 0.10		0.7 to 1.5	
July 10-12/07				
Trip Blank Water Sent to Field 7/9/07	0.0188	U	0.188	U
Average, Trip Blanks	0.0282		0.2219	
Field Blank Water Sent to Field 6/28/07	NA		0.188	U
Average, unfiltered blanks, n = 7	0.024		0.241	
Average, filtered blanks, n = 7	0.026		0.224	
Achieved Detection Limit	0.0159 0.0188		0.188	
Samples				
Unfiltered, mean +/- S.D.	0.153 +/- 0.173		3.72 +/- 5.75	
Filtered, mean +/- S.D	0.082 +/- 0.061		1.32 +/- 0.48	
Low samples, filtered, range	0.02 to 0.05		0.6 to 1.3	

Second Report on Interlab Comparisons and Quality Control/Quality Assurance Data from laboratories participating in the Penobscot Mercury Study

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September 2008

Contents

I. General description of the Quality Assurance/Quality Control (QA/QC) Program for the Penobscot Mercury Study

"Quality control" refers to *procedures that are done on a regular basis* in laboratories to determine the precision and accuracy of the methods used. For mercury and methyl mercury analyses, these procedures typically include the analysis of certified reference materials, internal standard additions of mercury or methyl mercury, measurement of the amount of trace mercury in reagents used in mercury analyses, determination of the precision of duplicate analyses, etc. In addition to *analytical* quality control procedures, there are also *field sampling* quality control procedures. For samples that will be analyzed for mercury, this includes verification of sampling handling techniques through handling of blank water in the same way that sample water is handled, and by taking replicate samples from the same site.

"Quality assurance" refers to a planned system of *review procedures* conducted by *personnel not directly involved* in the laboratory analysis of samples. It also refers to the *reporting system* by which the project laboratories provide information on the routine technical procedures that are used in "quality control". A good reporting system is complete and transparent, i.e., the reviewer can easily discern if standards, duplicates, reference materials, and recovery procedures are being tested out on a routine basis, and if the results of these procedures are within acceptable limits. Acceptable limits can be defined by agencies such as the EPA, by the analytical laboratory(s), or agreed upon between the client and the laboratories.

The two laboratories that routinely analyze samples for the Penobscot Mercury Study are Battelle Marine Sciences Laboratory in Sequim, Washington, and Flett Research Ltd. in Winnipeg, Manitoba. These two laboratories have achieved general accreditation by appropriate national agencies (the National Environmental Laboratory Accreditation Program in the U.S. and the Canadian Association of Environmental and Analytical Laboratories).

A third laboratory, run by Dr. Holger Hintelmann at Trent University in Trent, Ontario, has participated in inter-laboratory comparisons. Dr. Hintelmann is widely acknowledged to be an expert in the field of mercury analytical techniques.

The types of samples taken for the Penobscot Mercury Study include water, sediments, fish and other biota.

The goals of the QA/QC program for this Study are:
1. To monitor the quality control data provided by each laboratory for compliance with the standards set for accuracy and precision, for each type of analyses.
2. To monitor the results of tests of the field handling procedures for mercury samples.
3. To ensure that interlab comparison exercises for mercury in water, sediments, and tissues is carried out at least once a year.
4. To make recommendations to the laboratories, the field crew, and the study leaders regarding any needed improvements in sampling or analytical procedures.

II. Review of Quality Control Procedures and Transparency

The two laboratories that carried out routine analyses of Penobscot Mercury Study samples were Battelle Marine Sciences Laboratory in Sequim, Washington, and Flett Research Ltd. in Winnipeg, Manitoba. Details of the methods used were available, and are included in the Appendices of this report. Both of these laboratories reported their quality control data in a suitably transparent manner, and included these QC data with each set of analytical results provided to the Study. The quality control data included the results of analysis of a reference material suitable to the type of sample, e.g., when sediments were being analyzed, the reference material was a sediment and when biota tissue was being analyzed, the reference material was dogfish or lobster hepatopancreas. The reference materials used for each method are summarized in Appendix B. Also included were the results of spike matrix analyses, where a known spike of mercury or methyl mercury was added to a subsample of the material being analyzed, and the recovery of this known spike was determined. In addition, duplicate analyses were done and reported. Reagent blanks were analyzed for mercury or methyl mercury, depending on the analysis being done. If these quality control measurements were not within the guidelines established, the laboratory repeated the analyses.

In addition to the results for interlab comparison from both laboratories, the analytical reports on routine Penobscot Study samples were also made available to this reviewer, and were reviewed on a regular basis. These reports included results for water, sediment, and biota.

Field sampling records were also made available for review by Normandeau Associates, including the chain of custodyrecords. These were checked against records kept by the analytical laboratories for any problems that might occur in identifying samples sent from the field. Normandeau also made available a copy of their Standard Operating Procedure, with descriptions of the field sampling procedures to be followed. These were reviewed, and some suggestions were made on the procedures for taking blank samples. These suggestions were incorporated.

Overall, it is important to note that both analytical laboratories and Normandeau Associates have made available for review all information that was requested. This transparency in procedural details and in quality control data is essential to proper review, and there have been no problems in this area.

III. Water Sampling and Analyses

Samples were collected in the field by Normandeau Associates. personnel. They were shipped to the analytical laboratory in coolers by courier. Laboratory procedures on receipt of samples were to check the cooler temperature to ensure that it was within the optimal temperature range for unpreserved samples (4±2°C), and to preserve the samples within 48 hours. Freshwater and brackish water were preserved with HCl, added to a final concentration of 0.5%. Seawater samples were preserved with H_2SO_4, added to a final concentration of 0.2%. Total Hg samples were analyzed within 90 days and MeHg samples within 180 days. During Phase I of the Study, routine water analyses were done at Battelle Laboratory.

Analytical precision—analysis of duplicates

The precision of analyses of mercury in water was determined by carrying out duplicate analyses on single samples. (Note: analytical duplicates are different from field replicates, where two different samples are taken from the same site, and sample to sample differences will play a role in the variability of the results. For analytical duplicates, only differences in the analytical operations affect the precision.) All routine water analyses for the Penobscot Study were done at Battelle Laboratory, and the analytical duplicate data are from this lab.

The relative per cent difference (RPD) for each pair of duplicate analyses was calculated (% RPD = ((Sample A- Sample B)/ (average of A & B))*100. The average RPD's for both total mercury and methyl mercury duplicates done during both the May 2007 and July 2007 sampling periods (Table 1) were well within the recommended limit of 24% (EPA Method 1631).

Table 1. Precision in analytical duplicates of unfiltered water samples. Analyses done at Battelle Marine Sciences Laboratory. The recommended EPA limit is +/- 24%.

Sampling Period (2007)	**Total Mercury**		**Methyl Mercury**	
	Average %RPD	**n**	**Average %RPD**	**n**
May 31-June 2	**4.4** +/- 3.2	9	**10.3** +/- 11.2	**8**
July 10-12	**5.2 +/- 3.6**	**12**	**8.9 +/- 7.9**	**14**

Sample handling--Field blank results

The purpose of field blank measurements was to check for contamination of water samples that can occur through contact of the water or sample bottles with 1) the personnel doing the sampling, 2) the sampling equipment, 3) the immediate surroundings, or 4) the inside the coolers in which samples were stored. The general approach was for the analytical laboratory to send "blank water" (water that has undetectable mercury) to the field, where the field crew carried out the same handling procedures with this blank water as are used for handling samples. These "field blank" samples are then analyzed to see if any contamination of the blank water has occurred during this handling. In addition, some bottles of blank water were shipped to the field site but never opened, and shipped back to the laboratory. These blanks are called "trip blanks". They reflect contamination that can occur simply through diffusion of elemental mercury through the Teflon or polypropylene, and/or on opening the bottles in the laboratory on return.

During Phase I, field blanks were part of the regular Penobscot Mercury Study sampling program, and were done usually at a frequency of every fifth sampling site. In 2007, there were two periods of water sampling (May 31-June 2, and July 10-12). Blank water was supplied by Battelle MSL, and mercury analyses were done by Battelle. Field procedures were carried out by Normandeau Associates at field sites on the Penobscot River.

Field Blanks--Methyl mercury in water. Field blank results for methyl mercury in water were acceptable in 2006 (previous report) and also in 2007 (Table 2 below). MeHg was undetectable (<0.0188ng/L) in the blank water sent to the field. After this water was passed through the sampling apparatus (unfiltered blanks), MeHg was just above detection (0.019 to 0.024 ng MeHg/L, Table 2). When this water was passed through both the sampling apparatus and the filter (filtered blanks), MeHg was also just above detection (0.018 to 0.026 ng MeHg/L, Table 2).

For methyl mercury, the field blank concentrations in May were below or just above the detection limit. This is the desired outcome for MeHg field blanks. In July, however, blanks had measureable MeHg. This is surprising, as MeHg is not generally present in sufficient quantities to cause contamination. The trip blanks also showed measureable MeHg (Table 2), so field procedures may not have been the problem. Field blank levels should continue to be watched in the future.

While the MeHg concentrations measured in the blanks were very low, blank concentrations can not be disregarded in data analysis. In regular samples taken in 2007, the average MeHg concentrations were 0.08 to 0.15 ng MeHg/L, depending on the sampling period, and whether the sample was filtered or unfiltered (Table 2). Where the sample concentration is at least 5 times the blank concentration, the blank correction might not be important. However, for samples at the low end of the concentration range (0.02 to 0.10 ng MeHg/L), there may need to be some correction for, or at least acknowledgment of, the concentration of MeHg that could be due simply to sample handling.

Field Blanks--Total mercury in water. On both sampling occasions in 2007, field blank results for total mercury in water were acceptable (Table 2). This had also been the case in the last sampling period of 2006. Earlier in 2006, however, some of the THg field blank results were higher than desired. However, it could not be resolved whether the high THg results were caused by contaminated blank water, or by faulty field procedures, because there were some uncertainties in the record with respect to the mercury level of the blank water sent to the field from Studio Geochimica in early 2006. In the last sampling period of 2006, blanks were done using water sent by Battelle Laboratories, and the field blank results were well within the acceptable range (< 0.5 ng THg/L, previous report).

Total mercury is more likely than MeHg to show up as a contaminant due to sample handling, but the total mercury field blanks demonstrated good handling procedures. Blank values were about 0.2 ng HgT/L, which is below the recommended limit of 0.5 ng HgT/L (EPA Method 1631). Blank values were well below the average values of samples (1 to 4 ng THg/L), and also below the lowest sample values (0.5 to 0.6 ng HgT/L). As with MeHg samples, an acknowledgement of blank contributions is recommended for these very lowest THg sample values.

Trip Blanks for Total and Methyl Mercury in Water. Trip blanks were done in July, 2007, and analyzed for total mercury. These trip blanks had approximately the same increases in MeHg and THg, compared to the original blank water, that the field blanks showed (Table 2). This shows that field procedures added very little in the way of contamination, compared to unavoidable contamination that occurs during shipping and handling in the laboratory.

Table 2. Results for field blanks done in association with water sampling. Values with * are below detection limits (0.0188 ng MeHg/L and 0.188 ng THg/L).

Sampling Period		MeHg ng/L	THg ng/L
May 31-June 2/07	Average, unfiltered blanks, n = 5	0.019	0.213
	Average, filtered blanks, n = 5	0.018	0.192
	Blank Water Sent to Field 4/23/07	0.0188*	0.188*
	Water Sample results (for comparison to blanks)		
	Unfiltered, mean +/- S.D.	0.143 +/- 0.062	2.95 +/- 0.90
	Filtered, mean +/- S.D	0.112 +/- 0.056	1.945 +/- 0.631
	Low samples, filtered, range	0.02 to 0.10	0.7 to 1.5
July 10-12/07	Average, unfiltered blanks, n = 7	0.024	0.241
	Average, filtered blanks, n = 7	0.026	0.224
	Trip Blank Water Sent to Field 7/9/07	0.0188*	0.188*
	Average, Trip Blanks	0.0282	0.2219
	Blank Water Sent to Field 6/28/07	NA	0.188*
	Water Sample Results (for comparison to blanks)		
	Unfiltered, mean +/- S.D.	0.153 +/- 0.173	3.72 +/- 5.75
	Filtered, mean +/- S.D	0.082 +/- 0.061	1.32 +/- 0.48
	Low samples, filtered, range	0.02 to 0.05	0.6 to 1.3

Overall, the field blank results demonstrated no obvious problems in field procedures, with respect to contamination of samples by shipping or sampling procedures. However, the blank results do need to be taken into account when using results from samples when THg or MeHg concentrations are very low.

Combined sampling and analytical precision-- Field replicates

Field replicates are independently taken samples, as opposed to analytical duplicates. This means that two separate bottles were taken at each site, for each of the mercury analyses that would be done later (filtered methyl mercury, unfiltered methyl mercury, filtered total mercury, and unfiltered total mercury).

The purpose of the replicate sampling program was to determine the variation that is inherent in taking a sample from waters where currents may cause spatial heterogeneity in surface water concentrations. Good replication is an indication of good sample handling, but it should also be kept in mind that there is natural heterogeneity in water systems, especially for particulates. Thus, it is expected that the reproducibility for filtered waters will be better than for unfiltered waters, where different concentrations of particulates may introduce differences in total concentrations of mercury in samples. Measurements of the mass of particles (total suspended solids, or TSS) was included in this aspect of the study.

Replicate samples of water for mercury analyses were taken in the 5 Phase I sampling periods, from late August, 2006 through July 2007. In addition, for the last 3 sampling periods (October 2006, May 2007 and July 2007), replicate samples were taken for total suspended solids (TSS). Field replicate results for the 2006 water samplings were reported earlier (July 2007), but are included here so that overall study trends can be examined.

In each sampling period, 28 to 38 replicates (pairs) of samples were taken for each analysis. The relative percent difference (RPD = difference between the two replicates divided by the average of the two replicates) was calculated for each pair (Table 3).

Total Mercury. Sample replication for both filtered and unfiltered total mercury was very good, with the relative percent difference (RPD) in replicate samples averaging from 3 to 13% (Table 3). These results are within the EPA guideline of less than 20% RPD in field replicates.

The RPD's for unfiltered samples were not consistently greater or smaller than the RPD's for filtered samples (Table 3).

Table 3. Relative percent difference (RPD) for pairs of replicate water samples analyzed for filtered total mercury and for replicate samples analyzed for unfiltered total mercury.

	THg filtered water Field Replicates		**THg unfiltered water Field Replicates**	
	Average RPD +/- Std Dev	**n**	**Average RPD +/- Std Dev**	**n**
Sep 6-11/06	11.4 +/- 11.1%	33	6.4 +/- 7.5 %	32
Sep 27-Oct 5/06	10.5 +/- 13.0 %	37	5.0 +/- 5.5 %	37
Oct 22-25/06	9.6 +/- 8.1%	35	10.3 +/- 9.0 %	34
May 29-June 1/07	6.7 +/- 8.6 %	35	5.9 +/- 8.1%	35
July 10-12/07	4.1 +/- 3.0 %	35	11.4 +/- 14.9 %	35

Methyl Mercury. The variability for replicate water samples taken for filtered and unfiltered methyl mercury (Table 4) was greater than for filtered and unfiltered total mercury (Table 3). This is somewhat expected because the methyl mercury concentrations are much lower than total mercury concentrations, and more difficult to measure analytically (*analytical* duplicates had RPD's of 9-10% for MeHg in water, compared to 4-5% for THg). However, field replicates showed much greater variation than can be accounted for by analytical variation alone (field replicate RPD's averaged 19 to 23% for filtered samples, and 12 to 33 % for unfiltered MeHg samples, Table 4). The variability is probably not due to contamination, as MeHg is not very abundant in air or on people handling samples. The most likely reason for the variability is heterogeneity in the water being sampled.

The average RPD's were within the range recommended by the EPA (less than 35%). However, some individual replicates obviously fell outside this range, especially for unfiltered MeHg samples (Table 4).

Table 4. Relative percent difference (RPD) for pairs of replicate water samples analyzed for filtered methyl mercury and unfiltered methyl mercury. Analyses by Battelle MSL.

	MeHg filtered water Field Replicates		**MeHg unfiltered water Field Replicates**	
	Average RPD +/- Std Dev	n	Average RPD +/- Std Dev	n
Sep 6-11/06	19.2+/- 30.5 %	38	28.1 +/- 23.8 %	34
Sep 27-Oct 5/06	23.0 +/- 22.5 %	36	25.8 +/- 24.8 %	37
Oct 22-25/06	21.8 +/- 15.2 %	28	33.4 +/- 23.8 %	29
May 29-June 1/07	15.6 +/- 15.8 %	35	12.2 +/- 8.2 %	35
July 10-12/07	18.5 +/- 21.5 %	35	15.9 +/- 15.0%	35

Total Suspended Solids (TSS). TSS is a direct measurement of concentrations of particulate matter in a water sample. In the Penobscot Study, these measurements are used, together with the difference between unfiltered and filtered mercury concentrations, in the calculation of the concentrations of mercury in particles.

Samples were taken for measurement of TSS in the October/06, May/07, and July/07 sampling periods. Blanks are not an issue for this measurement, but reproducibility in waters where turbidity may be heterogeneous is a concern. The relative percent difference for replicate pairs of samples averaged 10.6 to 16.7% in the different sampling periods (Table 5). There are no set criteria for this; rather the RPD's of replicates is useful as an indicator of the variability that must be taken into account in using TSS measurements.

Table 5. Relative percent difference (RPD) for replicate water samples analyzed for total suspended solids. Analyses by Battelle MSL.

	Total Suspended Solids, water Field Replicates	
	Average RPD +/- Std Dev	n
Oct 22-25/06	16.7 +/- 14.1%	34
May 29-June 1/07	10.6 +/- 8.0%	35
July 10-12/07	12.9 +/- 11.6%	35

Comparison of RPD's for different analyses, and over time. The RPD's for replicate pairs of samples were always highest in the results for methyl mercury in water (Figure 1). The RPD's were lowest for THg, with TSS in between.

During the two sampling seasons of Phase I, some trends over time can be discerned. For total mercury, reproducibility in sampling and analyses was consistent throughout all periods. Reproducibility for TSS was also consistently good, considering that particulates can be quite variable in water samples. For methyl mercury, however, reproducibility was slightly better in the last two sampling periods (Figure 1). Taken all together, the data indicate that precision in sample handling and analyses has improved slightly in 2007.

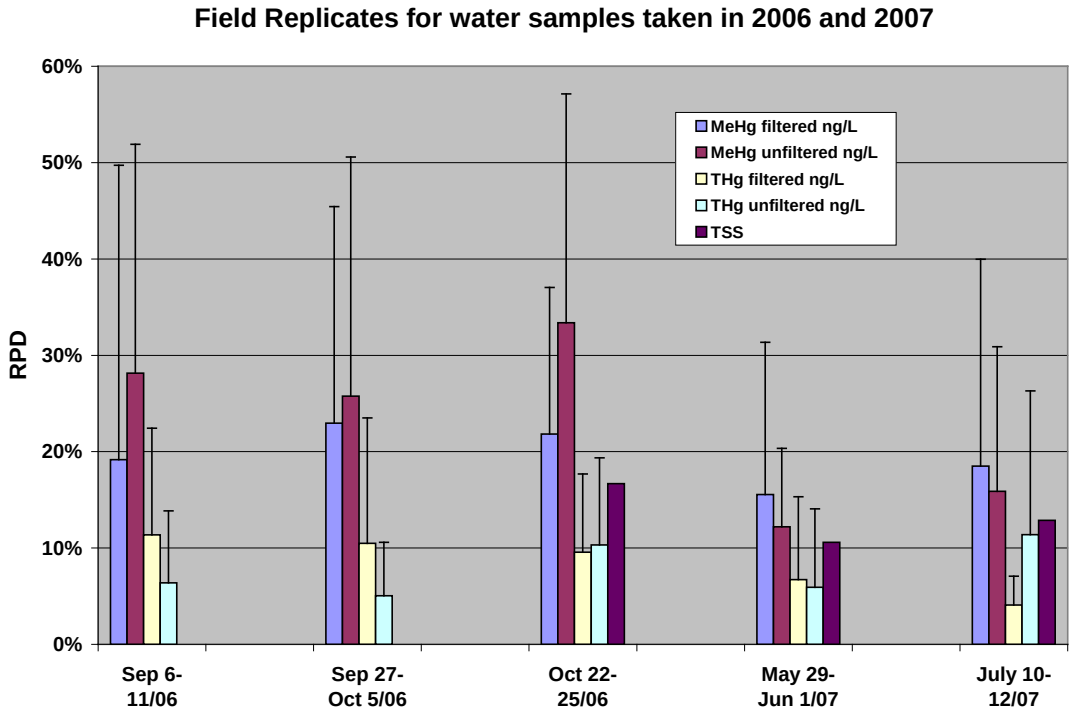


Figure 1. Average relative percent difference in replicate samples taken for total mercury (filtered and unfiltered), methyl mercury (filtered and unfiltered) and total suspended solids. Bars show the mean values, and the vertical lines show 1 standard deviation from the mean.

Interlab comparison on measurement of mercury in water, 2007

In May and July 2007, filtered water samples were taken from sites OV5 (freshwater) and OB2 (estuarine), and sent to the three laboratories that participated in the interlab comparison for measurement of both total and methyl mercury. All three labs used slight variations on EPA Method 1631e for total mercury in water, and EPA Method 1630 for methyl mercury in water. Flett Research and Battelle used Cold Vapor Atomic Flourescence Spectroscopy (CVAFS) for mercury detection, while Trent U. used Isotope Dilution Mass Spectroscopy (IDMS).

Total Mercury in Water. There was very little variability in the results for total mercury in water within each lab (Table 6). The variation among the labs was also small (Table 6, Figure 2), with the % standard deviations on the mean result obtained by all three labs were only 5 to 14% (calculated from data in last column of Table 6).

Table 6. Interlab Comparison results for total mercury in water, 2007.

Average THg ng/L +/- Std Dev.				
	Battelle MSL	**Trent University**	**Flett Research**	**All Labs**
OV5 May	2.65 +/- 0.05	2.21 +/-0.06	2.21 +/- 0.04	2.35 +/- 0.25
OB2 May	1.86 +/- 0.04	1.68+/- 0.07	1.79 +/- 0.09	1.78 +/- 0.09
OV5 July	2.03+/- 0.19	1.62+/- 0.02	1.59 +/- 0.15	1.75 +/- 0.25
OB2 July	1.34 +/- 0.07	1.20 +/- 0.04	1.14 +/- 0.06	1.23 +/- 0.10

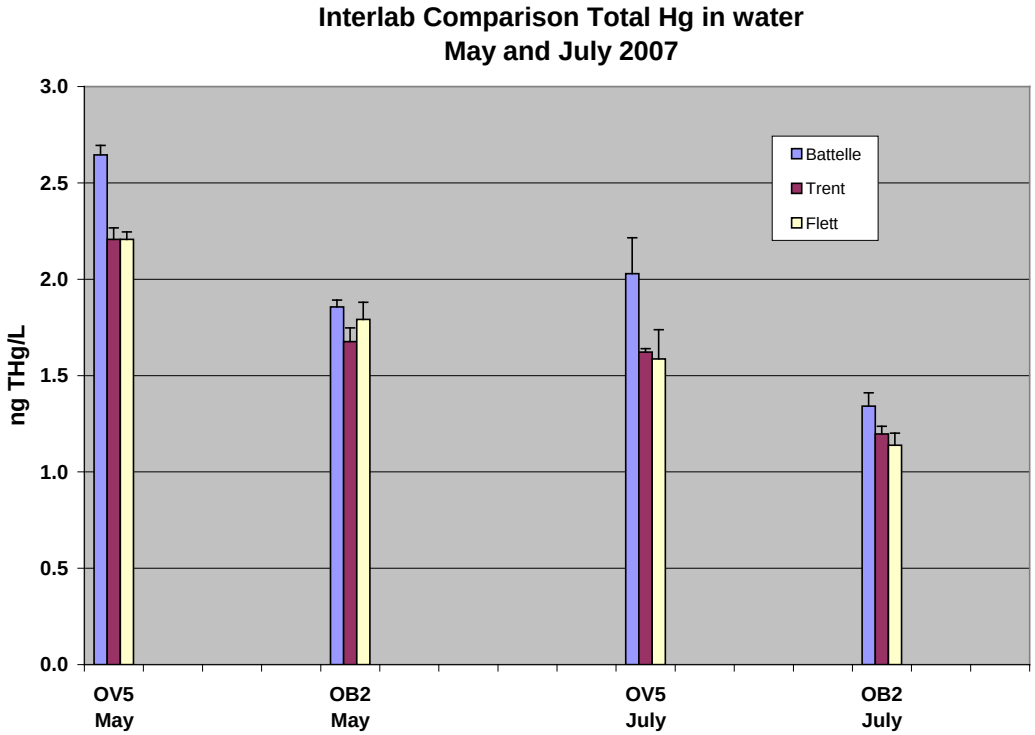


Figure 2. Average THg concentrations for each lab, plus the standard deviation for each lab's results, for the May and July sampling periods in 2007. The % standard deviations ranged from 5.1 to 14.1% of the mean values from all 3 laboratories.

A difficulty in interpreting the results of interlab comparisons where natural samples are used is that there is no "true" or "certified" value. However, the use of natural samples is necessary because components in water other than the mercury can affect the outcome of the analyses. A mathematical approach that has been developed for this purpose is the calculation of "z-scores". The value obtained by averaging the results from all three labs (last column, Table 6) is called the "reference value". The z-score calculation is made to quantify how far away each lab was from this reference value, with a z-score of "1" equal to a 5% difference, "2" equal to a 10% difference, and so on. The equation is

$$Z = (\text{lab result-reference value})/0.05$$

One approach to the criterion for acceptability is to use the same degree of difference allowed for replicate water samples, with the idea being that each lab's result is one replicate, and the reference value is the other replicate of the pair. For total mercury in water, this is 25%. In the format used here, this would equal a z-score of 5 or less. All of the z-scores were within the criterion (Table 7).

Table 7. Interlab comparisons for total mercury in water, z-score for each laboratory. Acceptable z-scores are ≤ 5.

	Battelle MSL		**Trent U.**		**Flett Res. Ltd.**	
	Average THg ng/L	z-score	Average THg ng/L	z-score	Average THg ng/L	z-score
OV5 May	2.65	2.49	2.21	1.24	2.21	1.24
OB2 May	1.86	0.92	1.68	1.11	1.79	0.19
OV5 July	2.03	3.24	1.62	1.42	1.59	1.82
OB2 July	1.34	1.89	1.20	0.47	1.14	1.43

Methyl mercury in water. The results from the interlab comparison on methyl mercury in water were not as complete as for total mercury in water. All 3 labs participated successfully in the May inter-comparison, but only two labs were able to complete the July inter-comparison. In the July sampling, difficulties with sample contamination and a laboratory error made the results from one lab not useable. Fortunately, the lab that does the routine water analyses completed both exercises and the results were satisfactory, if more variable than for total mercury.

Variability within each lab was very low (Table 8). The standard deviations on the average value for all labs were 0.6 to 38% of the averages for each sampling site and date (calculated from the last column in Table 8).

Table 8. Interlab comparison results for methyl mercury in water, 2007.

ng MeHg/L Average +/- Std. Dev.				
	Battelle MSL	**Trent U.**	**Flett Research**	**All Labs**
OV5 May	0.20 +/- 0.02	0.18 +/-0.00	0.15 +/- 0.07	0.18 +/- 0.03
OB2 May	0.11 +/- 0.01	0.11 +/- 0.01	0.10 +/- 0.01	0.10 +/- 0.03
OV5 July	0.12 +/- 0.01	0.12 +/- 0.01	*	0.12 +/- 0.00
OB2 July	0.06 +/- 0.02	0.03	**	0.05 +/- 0.0.02

* These results not useable because of methods error in lab

** These results not useable because of obvious contamination in sample bottles

The average results for each lab were not in as close agreement for methyl mercury in water (Table 9; Figure 3) as for total mercury in water (Table 7, Figure 2). This is expected and is reflected in the greater RPD permitted by the EPA for methyl mercury replicates (35%, compared to 25% for total mercury). It should also be noted that the levels of these concentrations were low, close to the method limit of 0.14 ng/L established at Flett Research for reliable quantification.

In evaluating z-scores for MeHg in water, the criterion for acceptability was ≤ 7, reflecting the EPA acceptability level of 35% for RPD between replicate samples for MeHg (1 z-score unit is a difference of 5%). With this criterion, all of the interlab results were acceptable (Table 9).

Table 9. Laboratory intercomparison for methyl mercury in water, z-scores for each lab.

	Battelle MSL		Trent U.		Flett Research Ltd	
	Average MeHg ng/L	z-score	Average MeHg ng/L	z-score	Average MeHg ng/L	z-score
OV5 May	0.20	2.76	0.18	0.69	0.15	3.45
OB2 May	0.11	2.99	0.11	3.21	0.07	6.19
OV5 July	0.12	0.08	0.12	0.08	*	
OB2 July	0.06	5.32	0.03	5.32	**	

* These results not useable because of methods error in lab

** These results not useable because of obvious contamination in sample bottles

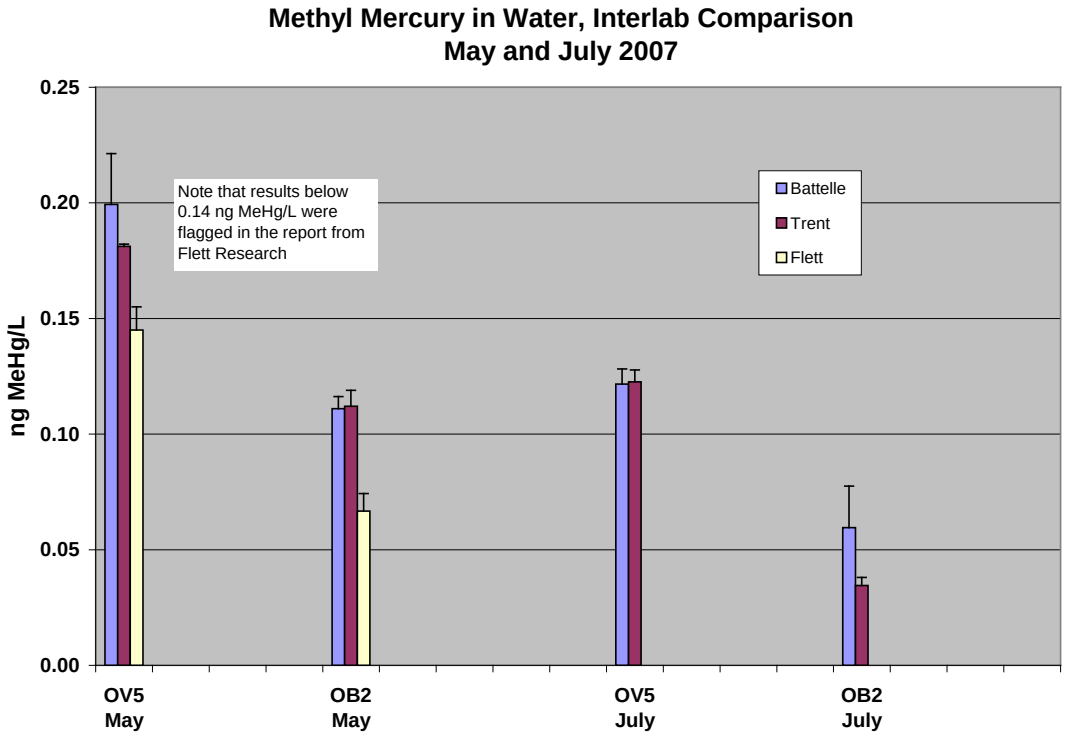


Figure 3. Results of laboratory intercomparison, methyl mercury in water, 2007. Bars show the mean values, and the vertical lines show 1 standard deviation from the mean.

Recommendations with respect to water analyses

There were no problems that surfaced with respect to field handling or analyses of mercury or methyl mercury in water. Several recommendations are made in order to ensure continued good performance and data analysis:

- There needs to be continued high vigilance on field sampling procedures, as a common hazard in trace metal sampling is that field crew personnel become overconfident when there have been no problems! I recommend that an experienced mercury scientist review procedures on site in 2008.
- Concentrations of both methyl mercury and mercury in field blanks should continue to be monitored closely.
- The next lab inter-comparison should be done as soon as water sampling commences in 2008, with special attention to MeHg blanks and samples. Sites chosen should include one site with higher MeHg concentrations than at the two sites used in the 2007 exercise. This would provide comparison of results at concentration levels where the results are generally more reliable for any lab that is carrying out analyses of methyl mercury in water.
- When analyzing the data for water samples, the blank results (about 0.02 ng/L for MeHg and about 0.2 ng/L for THg) should be taken into account, especially when sample concentrations are low (0.02 to 0.10 ng/L for MeHg in water, and 0.2 to 1.0 ng/L for THg in water).

IV. Sediment sampling and analyses

In the previous interlab exercise (2006), no problems were identified in the analyses of total mercury in Penobscot River sediments. However, the results for methyl mercury were quite different from the different laboratories, and this appeared to be related to methodological differences among the laboratories in 2006. These methods have since been extensively tested and investigated by the participating laboratories. Because of this need for methods testing for the methyl mercury analyses, and the lack of this need with respect to total mercury analyses, the QA/QC results for the total mercury in sediments are presented separately from the results for methyl mercury in sediments.

Sediment samples were collected in the field by Normandeau Associates, and were frozen immediately. For routine analyses in Phase I, frozen samples were sent to Battelle MSL in Sequim, WA. For methods testing, samples were sent to Battelle, to Flett Research Ltd., and to Trent University.

IV A. Total mercury in sediments

Analytical precision--Analytical duplicates.

Analytical duplicates were done on one in every ten sediment samples. The relative percent difference (RPD) was calculated for each pair of duplicates taken from a single sediment sample. While there are no set criteria, average RPD's of 4-7% (Table 10) can safely be described as low and indicate no difficulties.

Table 10. Relative percent differences in analytical duplicates for sediment samples collected May 30-June 1, 2007 and July 9-12, and analyzed for total mercury at Battelle Marine Sciences Laboratory.

	May 30-Jun 1/2007	**July 9-12/ 2007**
Average RPD	6.60%	**7.04 %**
Std Dev	4.18 %	**7.31 %**
n	**18**	**15**

Combined sampling and analytical precision--Field replicates.

Field replicates are sediment samples taken independently, at one site. Because two separate cores are taken, the relative per cent difference between samples is expected to be greater than for analytical duplicates (Table 10, above), where both samples came from the same core section. This was the case (Table 11, below). For sediments, contamination is not the same concern as for examining field replicates of water samples, because sediments contain much higher amounts of mercury. Rather, the primary usefulness of these sediment RPD's is

to provide a reference point for statistical expectations on the precision of core data, given the heterogeneity of sediments at a single sampling site. The RPD's found were not higher than expected for core to core variation.

Table 11. Relative percent differences between replicate surface sediment samples taken from two independently taken cores at a single sampling site and analyzed for total mercury.

	May 30-Jun 1/2007	**July 9-12/ 2007**
Average RPD	16.1 %	**30.8 %**
Std Dev	20.5 %	**39.7 %**
n	**20**	**15**

Interlab comparison—Total mercury in sediments.

Three laboratories participated in an inter-lab comparison in 2007. Sediments collected in May 2007 and July 2007 were sent to each lab. The results from the three labs were clearly in good agreement (Figure 4).

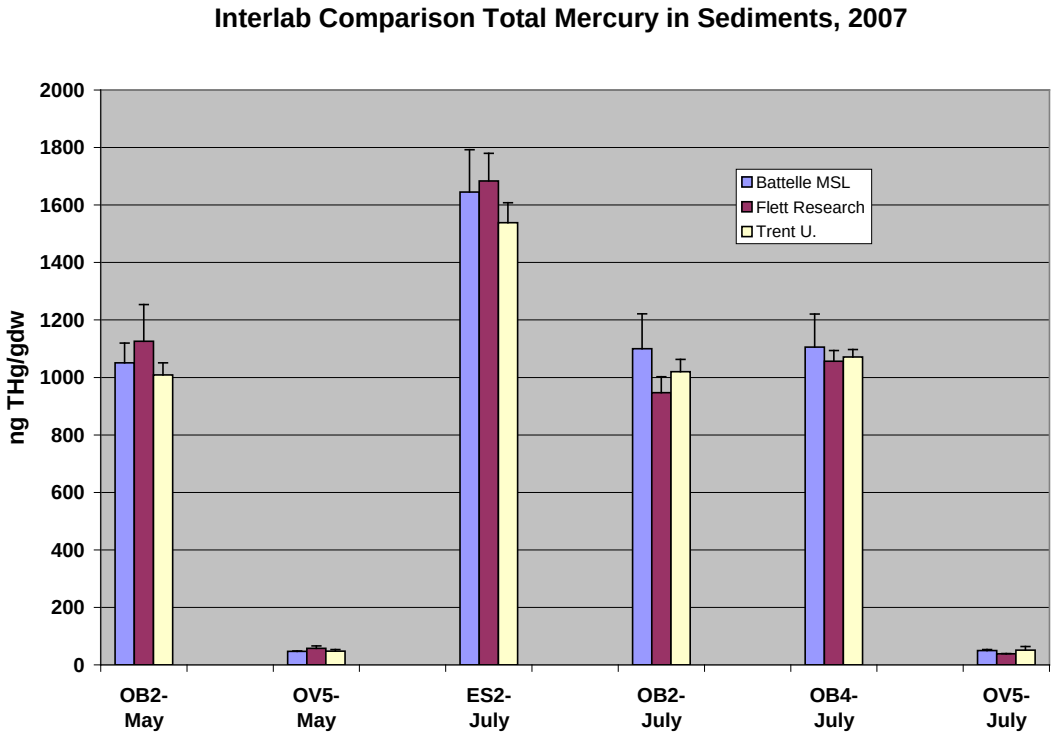


Figure 4. Results for inter-lab comparison on total mercury measurements in sediments. Sediments were collected at sites in the Penobscot River and estuary. Surface sediments (0-3 cm) were used.

The variation in results was small within each lab, as indicated by the standard deviations on the average result from each lab for each site (Table 12).

The z-score is a measure of the difference between each lab's average result, and the average result for all the labs, in units of 5% of the mean result. All z-scores were well within the acceptable range of 5 (Table 12).

Table 12. Numerical results from interlab comparison for total mercury in sediments, 2007.

	Battelle MSL		**Flett Research**		**Trent U.**	
Site	**Site Average ng THg/gdw**	**z-score**	**Site Average ng THg/gdw**	**z-score**	**Site Average ng THg/gdw**	**z-score**
OB2-May	1051 +/- 68.3	0.21	1126 +/- 127.7	1.21	1009 +/- 42.3	1.00
OV5-May	46.7 +/- 1.4	1.55	57.3 +/- 8.7	2.63	47.9 +/- 5.3	1.08
ES2-July	1645 +/- 147.3	0.28	1683 +/- 96.3	0.75	1538 +/- 69.3	1.03
OB2-July	1100 +/- 121.2	1.52	947 +/- 55.4	1.48	1020 +/- 42.8	0.05
OB4-July	1106 +/- 114.6	0.52	1056 +/- 37.0	0.40	1071 +/- 26.4	0.12
OV5-July	50 +/- 3.2	1.54	38 +/- 1.2	3.49	51 +/- 12.7	1.95

IV B. Methyl mercury in sediments

In the previous interlab exercise (2006), results for methyl mercury in sediments differed significantly among labs. Concentrations obtained by Battelle were lower, by as much as a factor of 2, compared to the other two labs. Battelle used extraction as the first step in their method, while Flett Research and Trent U. used distillation. At first, it was thought that the difference might be due to an analytical artifact previously identified for high mercury sediments when these sediments are analyzed using distillation. This artifact arises if some of the inorganic mercury in the sediments is chemically methylated during the distillation process. However, in the case of the Penobscot sediments, 1-2 % of total mercury would have to have been methylated in this way to account for the difference in the results, and previous measurements of this artifact have demonstrated that only 0.01 to 0.03% of inorganic mercury is typically methylated during the distillation step (Hintelmann et al, 1997). Thus, in 2007, a number of methods comparisons and tests were undertaken in order to investigate whether this difference in results from the different laboratories for Penobscot sediments was consistent, and if so, to make an informed choice as to methodology for the routine sediment analyses for methyl mercury.

In the examination of the sediment measurements, attention will be given first to precision of each of the two methods, for analytical duplicates done on a single sample, and for field replicates taken at a single site. Secondly, differences in the magnitude of the concentrations obtained using the two methods will be examined, with the objective of evaluating which method is most appropriate for Penobscot sediments.

Analytical precision--Analytical duplicates.

There are no EPA guidelines for acceptable RPD's for analytical duplicates in sediments (for MeHg in water the limit is 35%). Sediments are generally acknowledged to be more difficult to split reproducibility, because sediment samples are more heterogeneous than water. In any case, in samples taken for the Penobscot Mercury Study, the average RPD's for analytical duplicates were much lower than 35% (Table 13). Also, the precision of analytical duplicates for methyl mercury in sediment was similar for both the extraction and distillation methods. Thus, while giving different answers, one method gave just as consistent results as the other method.

Table 13. The relative percent differences between pairs of analytical duplicates (subsamples taken in the laboratory from one sediment core section). Methyl mercury analyses done at Battelle Marine Sciences Laboratory.

	Extraction		**Distillation**	
	Average RPD	**n**	**Average RPD**	**n**
May 30-Jun 1, 2007	6.0 +/- 5.9 %	6	10.9 +/- 10.8 %	16
Jul 9-12, 2007	10.5 +/- 10.9 %	5	8.6 +/- 8.4 %	16

Combined sampling and analytical precision--Field replicates.

Field replicates are samples taken from independently taken cores, at one site. As expected, the variation was greater for these replicates (Table 14, below) than for analytical duplicates taken from a single sample (Table 13, above). Both extraction and distillation methods showed similar degrees of variation in the results for field replicates (Table 14). There are no specific guidelines for acceptability in variation in field samples for methyl mercury in sediments. Rather, the statistics gathered on replicates is useful in making determinations of whether concentrations measured at different sites are any more different than concentrations measured within one site. This information is also necessary for comparing results on replicate samples analyzed by the two different methods, i.e., the differences need to be greater than the differences shown in Table 14 for one method in order to conclude that the two methods are actually giving different answers.

Table 14. Relative percent differences between pairs of field replicates in sediment samples analyzed for methyl mercury.

	Extraction		Distillation	
	Average RPD	n	Average RPD	n
May 30-Jun 1, 2007	44.7 +/- 29.2 %	5	37.9 +/- 37.0 %	15
July 9-12, 2007	---		35.3 +/- 43.0 %	15

Interlab comparison—Methyl mercury in sediments

Each participating laboratory used both the extraction and distillation methods to measure methyl mercury in the Penobscot interlab sediment samples. These methods differ in the first step, as indicated by their names. In one method, the methyl mercury was initially recovered from the sediments by solvent extraction , while in the other method the methyl mercury was recovered by distilling it out of the wet sediment sample. After this step, however, the methods were essentially the same—the recovered methyl mercury in the extract or distillate was ethylated, and mercury species were measured by Cold Vapor Fluorescent Atomic Spectroscopy (CVFAS) or by Isotope Dilution Mass Spectroscopy (IDMS).

When results were compared for the extraction method only, results from both Battelle MSL and Flett Research Inc. were almost identical in the May 2007 samples (Figure 5. In July, the results from Battelle were consistently higher (Figure 5). For all sites and samples, however, the interlab comparison results were good, with z-scores well within the criterion of 7 (Table 15).

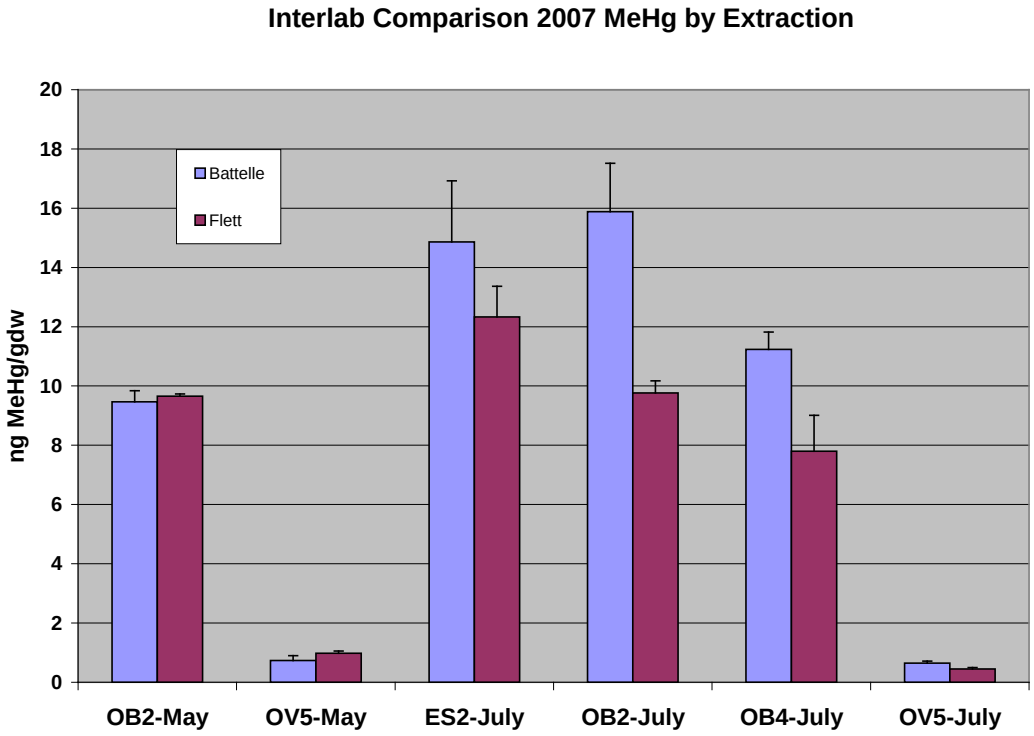


Figure 5. Methyl mercury in sediments, measured by extraction and CVFAS, at Battelle MSL and Flett Research Ltd. Bars show the mean values, and the vertical lines show 1 standard deviation from the mean.

Table 15. Interlab comparison results for methyl mercury in sediments measured by extraction on samples taken in 2007.

	Battelle MSL		Flett Research Ltd.	
Site	**Site Average ng MeHg/gdw**	**z-score**	**Site Average ng MeHg/gdw**	**z-score**
OB2-May	9.46 +/- 0.38	0.20	9.66 +/- 0.08	0.20
OV5-May	0.73 +/- 0.16	2.86	0.98 +/- 0.07	2.86
ES2-July	14.86 +/- 2.06	1.86	12.33 +/- 1.03	1.86
OB2-July	15.88 +/- 1.63	4.77	9.77 +/- 0.40	4.77
OB4-July	11.23 +/- 0.59	3.61	7.80 +/- 1.21	3.61
OV5-July	0.64 +/- 0.07	3.55	0.45 +/- 0.05	3.55

The same samples done by extraction, above, were also done by using distillation as the first step in the analyses (Figure 6). When results were compiled for this method, the Z-scores were within the acceptable range for all sites and dates, for all three labs (Table 16).

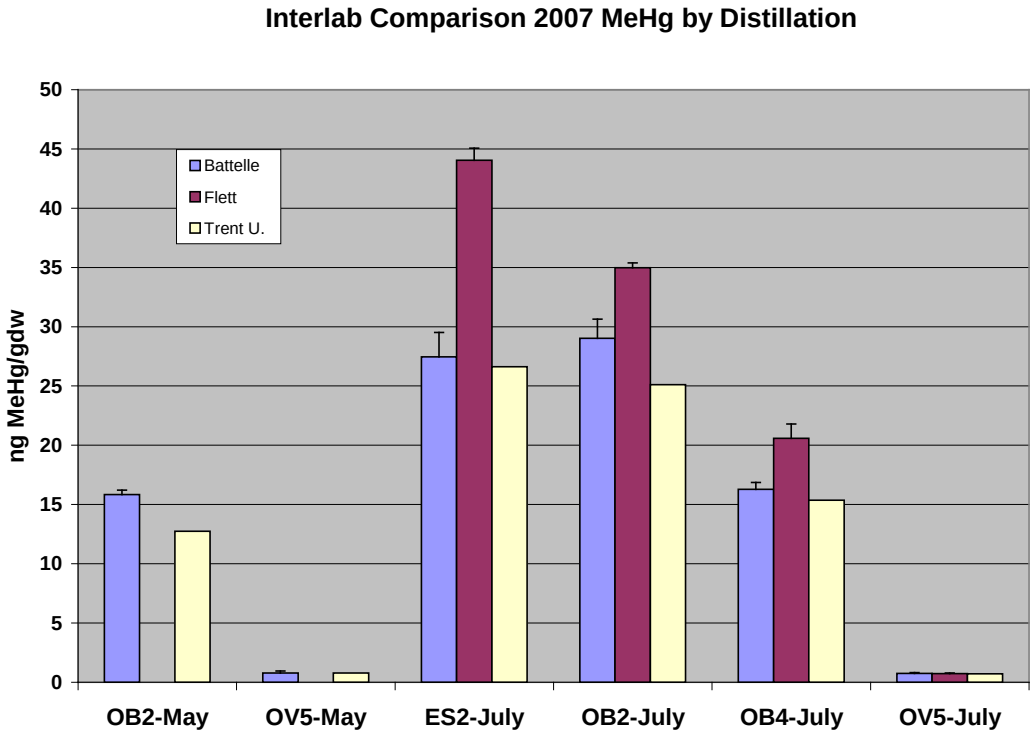


Figure 6. Methyl mercury concentrations measured in Penobscot River sediments by three laboratories, using distillation as the first step in the analysis. Bars show the mean values, and the vertical lines show 1 standard deviation from the mean.

Table 15. Interlab comparison of sediments analyzed for methyl mercury, with the first step being distillation. Samples were all surface sediments, collected in 2007.

	Trent U.		Battelle MSL		Flett Research Ltd.	
	Average (ng/gdw)	z-score	Average (ng/gdw	z-score	Average (ng/gdw	z-score
OB2-May	12.74 +/- 0.51	2.17	15.83 +/- 0.42	2.17		
OV5-May	0.79 +/- 0.11	0.08	0.78 +/- 0.12	0.08		
ES2-July	26.62 +/- 2.58	3.72	27.46 +/- 4.21	3.21	44.04 +/- 2.82	6.93
OB2-July	25.11 +/- 1.17	3.09	29.02 +/- 3.40	0.46	34.98 +/- 6.21	3.55
OB4-July	15.37 +/- 0.90	2.34	16.27 +/- 0.31	1.30	20.58 +/- 1.95	3.65
OV5-July	0.72 +/- 0.08	0.34	0.74 +/- 0.08	0.32	0.73 +/- 0.07	0.02

Thus, evaluations of precision, and interlab comparisons using only one method, did not show any data that would raise concerns. However, when all samples done by both extraction and distillation were compared, results using extraction were obviously lower than distillation. This was true in both 2007 (Figure 7) and 2008 (Figure 8), and when both

methods were done in the same laboratory, or in different laboratories. On average, the results using distillation were about twice as high as the results using extraction (Figure 9).

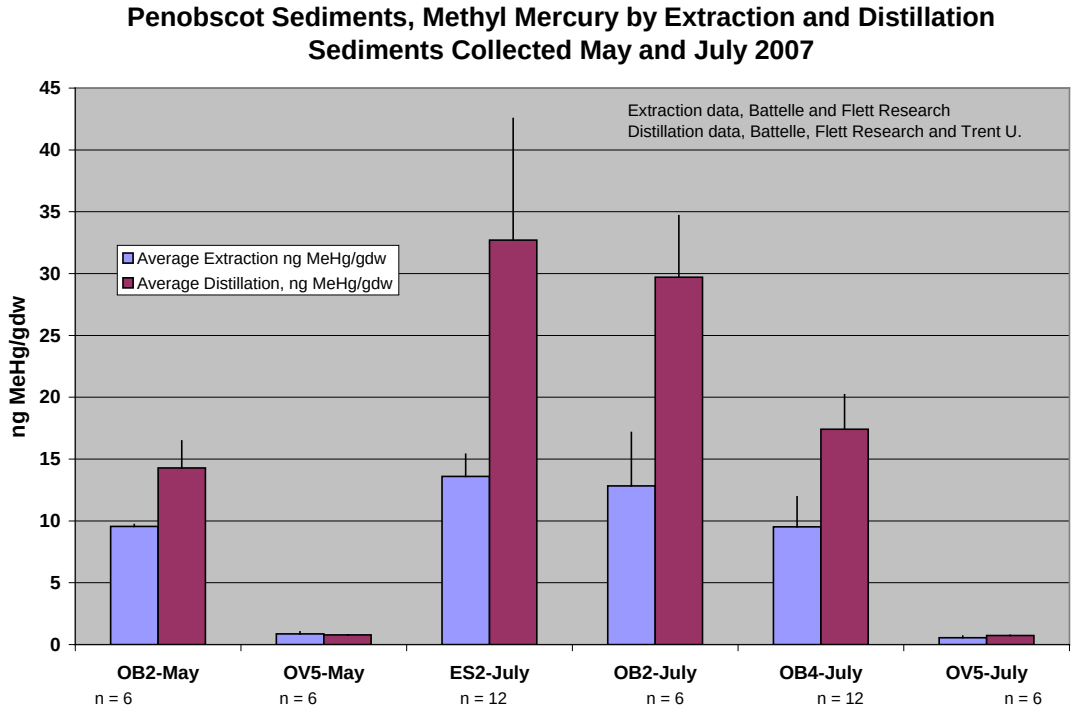


Figure 7. Comparison of methyl mercury results in sediments where the first step in analysis was extraction or distillation. Each bar is the average result, combining data from all labs, and the line above each bar shows 1 standard deviation on the mean.

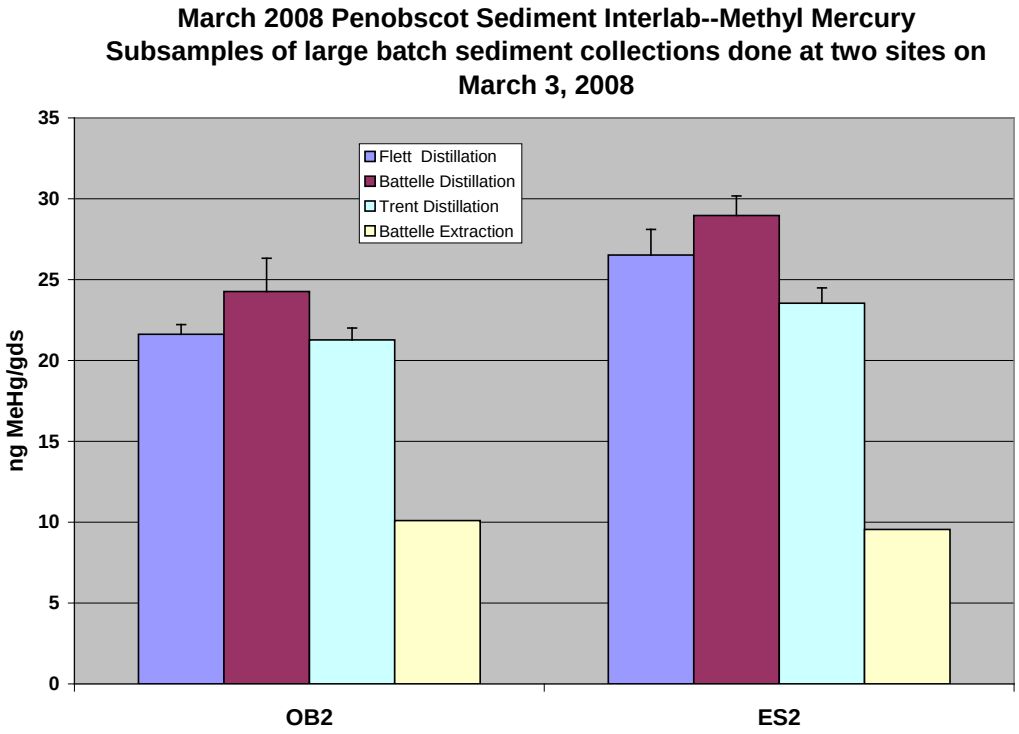


Figure 8. Methyl mercury measured in Penobscot River sediments, by two methods, in 2008. Battelle MSL did measurements by both methods; Flett Research Ltd. and Trent U. did measurements using distillation. Bars show the mean values, and the vertical lines show 1 standard deviation from the mean.

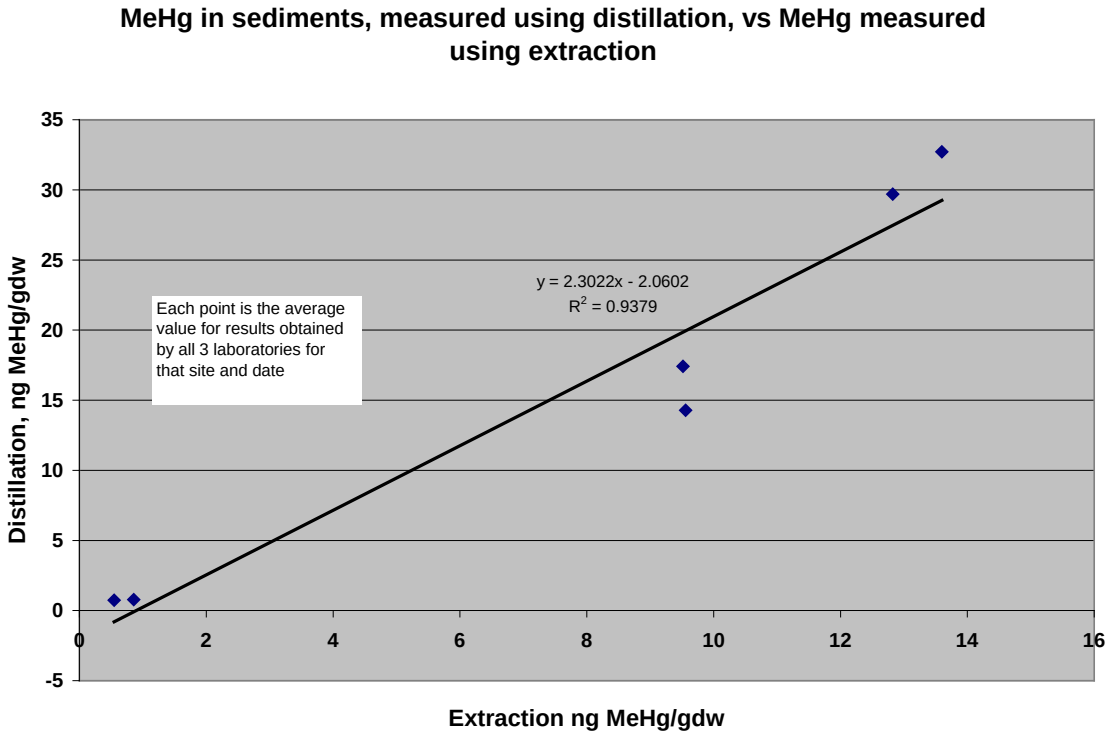


Figure 9. The average distillation result for MeHg in sediments at each site sampled for the interlab comparison in 2007, vs. the average extraction result for the same site.

The data above showed clearly that the distillation method consistently resulted in higher measurements of MeHg concentrations in Penobscot sediments than the extraction method. The magnitude of the difference (about 2X) was greater than would be expected for sample to sample variation (35 to 45%, Table 14). Thus, the next step was to determine if the higher results for distillation could be due to an artifact of the distillation method that has been identified in some situations. This artifact is the chemical methylation of a small portion of the inorganic Hg in the sample, producing MeHg that would not naturally be in the sample (Hintelmann et al, 1997).

One approach to evaluating the contribution of chemical methylation of inorganic Hg during distillation is to add isotopic inorganic Hg to the sample at the beginning of the distillation process. This was done at Trent U. in 2007, with the result that only 0.01 to 0.06% of the inorganic Hg added was converted to methyl mercury (Table 17). The average methylation rate was 0.03%, for 27 samples. Conversion of inorganic mercury isotope additions was also measured in 2008, with the average rate again being 0.03% (data not shown).

Table 17. Percentages of conversion of isotopic (^{200}Hg) inorganic Hg that was added to Penobscot sediments at the beginning of the distillation step. Analyses done at Trent University.

Sample ID	Station	Description	Date of Sampling	Ambient THg	^{200}Hg spike	^{200}MeHg	200Hg methylated (artifact)
				ng/gdw	ng	ng	%
MA-OV5-A	OV5	freshwater	11-Jul-07	0.72	3.1	0.000	0.00%
MA-OV5-A				0.72	31	0.003	0.01%
MA-OV5-B	OV5	freshwater	11-Jul-07	0.80	3.1	0.000	0.01%
MA-OV5-C	OV5	freshwater	11-Jul-07	0.64	3.1	0.001	0.03%
MA-OB2-A	OB2	tidal river	10-Jul-07	23.72	31	0.005	0.01%
MA-OB2-A				29.15	310	0.057	0.02%
MA-OB2-B	OB3	tidal river	10-Jul-07	24.63	31	0.010	0.03%
MA-OB2-B							
MA-OB2-C	OB4	tidal river	10-Jul-07	24.26	31	0.014	0.04%
MA-OB2-C				24.01	31	0.006	0.02%
MA-OB2-C				24.48	310	0.044	0.01%
MA-ES2-A	ES2	estuary	09-Jul-07	24.42	31	0.005	0.02%
MA-ES2-B	ES2	estuary	09-Jul-07	26.91	31	0.013	0.04%
MA-ES2-B				28.68	310	0.070	0.02%
MA-ES2-C	ES2	estuary	09-Jul-07	24.00	31	0.017	0.06%
MA-ES2-D	ES2	estuary	09-Jul-07	27.01	31	0.004	0.01%
MA-ES2-E	ES2	estuary	09-Jul-07	25.56	31	0.007	0.02%
MA-ES2-E							
MA-ES2-F	ES2	estuary	09-Jul-07	29.60	31	0.017	0.05%
MA-ES2-F				32.32	310	0.098	0.03%
MA-OB4-A	OB4	tidal river	09-Jul-07	14.60	31	0.021	0.07%
MA-OB4-B	OB4	tidal river	09-Jul-07	15.15	31	0.008	0.03%
MA-OB4-C	OB4	tidal river	09-Jul-07	16.36	31	0.016	0.05%
MA-OB4-D	OB4	tidal river	09-Jul-07	14.75	31	0.010	0.03%
MA-OB4-D				16.99	310	0.091	0.03%
MA-OB4-E	OB4	tidal river	09-Jul-07	13.71	31	0.006	0.02%
MA-OB4-E				13.37	31	0.001	0.00%
MA-OB4-E				16.73	310	0.095	0.03%
MA-OB4-F	OB4	tidal river	09-Jul-07	14.18	31	0.008	0.03%

In the isotopic method above, only a small amount of additional inorganic mercury is added to the sample, because the measurement method is very sensitive. A second approach to examining the chemical methylation of inorganic Hg during distillation is to add fairly large amounts of inorganic Hg, enough to measure chemically any increase in methyl mercury caused by these additions. This was done at both Battelle MSL and at Flett Research Inc.

Rates of conversion ranged from 0 to 0.09% of the added Hg (data not shown), which was similar to the isotopic approach (Table 17, above).

Could this small rate of chemical methylation of inorganic Hg in Penobscot sediment samples account for the higher concentrations of MeHg found with the distillation method, compared to the extraction method? This was examined by calculating the average increases in MeHg values obtained by distillation, compared to extraction, and dividing this difference by the total Hg concentration at each site (most of the total Hg in these sediments was inorganic Hg, as MeHg concentrations were very low, compared to THg concentrations, Table 18). These increases were 0.40 to 1.65% of total Hg, except in the case of OV5 sediments sampled in May 2007, where there was almost no difference in MeHg measured by the two methods (Table 18). Given the direct measurements of inorganic Hg conversion rates during distillation of Penobscot sediments (Table 17), as well as data from other studies on this phenomenon (0.030 to 0.036%, Hintelmann et al, 1997), it is unlikely that this type of conversion by itself could explain the much higher values obtained using distillation, compared to extraction, on Penobscot sediments (Table 18).

Table 18. Methyl mercury in sediments measured by using extraction or distillation as the first step at each site, and the difference in the two method results expressed as a % of total mercury in sediments at each site.

Site (2007)	Site average (all labs) Extraction, ng MeHg/gdw	Site average (all labs) Distillation, ng MeHg/gdw	Distillation minus Extraction, ng MeHg/gdw	Site average (all labs) ng THg/gdw	Distillation minus Extraction, divided by THg, %
OB2-May	9.56 +/- 0.14	14.29 +/- 2.19	4.73	1062	0.45%
OV5-May	0.86 +/- 0.17	0.78 +/- 0.00	-0.07	51	0.00%
ES2-July	13.60 +/- 1.79	32.71 +/- 9.82	19.11	1622	1.18%
OB2-July	12.82 +/- 4.32	29.70 +/- 4.97	16.88	1022	1.65%
OB4-July	9.52 +/- 2.43	17.41 +/- 2.79	7.89	1078	0.73%
OV5-July	0.55 +/- 0.14	0.73 +/- 0.01	0.19	46	0.40%

In summary, the distillation method for MeHg in sediments resulted in higher values for MeHg concentrations in Penobscot than did the extraction method, and the higher values could not be accounted for by measurements of chemical methylation during the distillation procedure. Thus, the extraction method did not appear to be recovering all the MeHg that was in the sediments, because the distillation method recovered much more. Clearly, the use of extraction underestimates the concentration of MeHg in Penobscot sediments.

Interestingly, all three labs used the same reference material (IAEA-405, an estuarine sediment widely used as a reference for mercury analyses), and analyses of this material by both methods gave results within the guidelines for recovery of MeHg from sediment samples. This is important because laboratories depend on analysis of reference materials to

alert them to potential problems in their measurement methods. Also, the initial comparison of extraction vs. distillation during the development of these methods for measurement of MeHg in sediments (Horvat et al 1993) did not show differences in results. Thus, the clear difference between the extraction and distillation methods for Penobscot sediments was not expected, and could only be discovered through the thorough methods comparison that was carried out.

The extensive comparison of the results from both methods on Penobscot sediments clearly showed that use of extraction on these sediments resulted in under-measurement of MeHg concentrations. There are very few other examples of studies where a large number of locations and sediment types have been investigated for possible differences in MeHg concentrations obtained by these two analytical methods. Most investigations use one method and a reference material. Therefore it is not possible to say whether Penobscot sediments are unique in the characteristic of under-measurement by extraction. What is clear is that methodological considerations are very important for measurement of MeHg in these sediments.

The rest of this section is concerned with the decision making process in choosing the best method for routine sediment analysis for methyl mercury in the Penobscot system. The extraction method was initially chosen for MeHg in sediments because many of the Penobscot samples are high in total Hg, and it was considered desirable to avoid the possibility that artifact conversion of inorganic Hg might occur during methyl mercury analyses. All samples taken in 2006 were analyzed by this method. After discussion with the analysts, the Study Panel, and the Project Leader, it was decided that distillation should be the method of choice for methyl mercury measurements on Penobscot sediment samples collected in after 2006. Also, while all work to date has shown that chemical methylation during distillation was very small, this should be continued to be monitored, to quantify the contribution of this chemical methylation on a continuing basis.

While it is usually not desireable to change methods during a study, the evidence that the extraction method must not be recovering all of the methyl mercury in the Penobscot sediment samples could not be ignored. In order to deal with the change in methods, and to retain the ability to compare 2006 data with samples collected later, it was also decided that 20% of samples done after 2006 would be done using both extraction and distillation, for year to year comparison. This decision resulted in the accumulation of a large number of samples done by both methods in 2007, and showed that the difference in results seen in the interlab comparison exercises continued consistently in later analyses. In May, 2007, results for MeHg in sediments by the distillation and extraction methods were significantly and linearly related to each other, with distillation results 2.4 times higher on average than extraction results (Figure 10, below). The same linearity was seen in July 2007, but with distillation averaging 1.8 times higher (Figure 11, below). This consistency means that results for samples done by only one method or the other can be compared approximately by using a factor of 2, with distillation results about 2 times greater than extraction results.

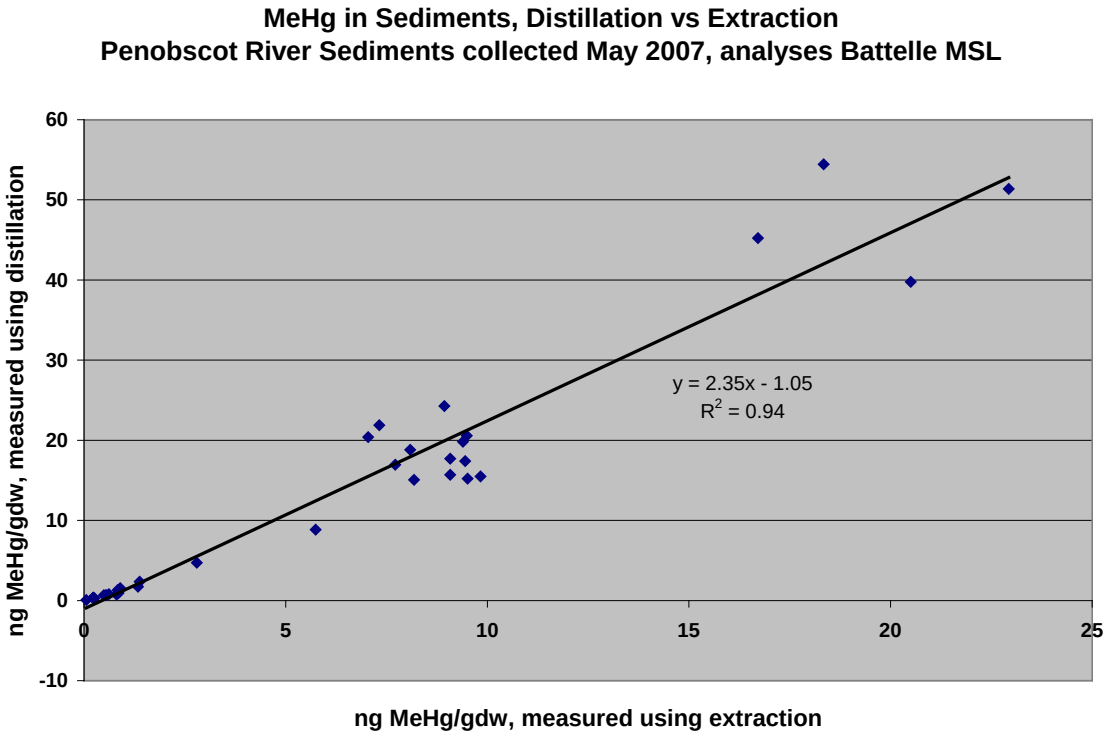


Figure 10. Comparison of MeHg concentrations measured in Penobscot River sediments collected in May 2007, and analyzed using both extraction and distillation as the first step in the analytical method.

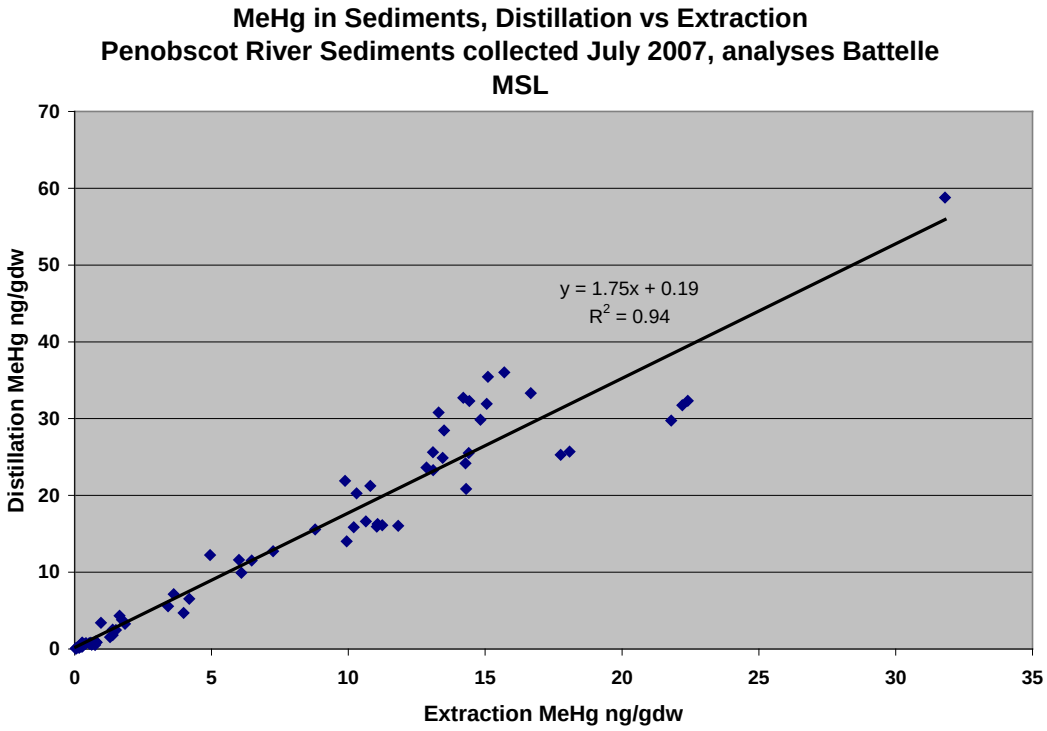


Figure 11. Comparison of MeHg concentrations measured in Penobscot River sediments collected in July 2007, and analyzed using both extraction and distillation as the first step in the analytical method.

Recommendations with respect to sediment samples

There were no changes recommended with respect to total mercury analysis. For methyl mercury analyses the following recommendations were made and have been carried out:

- Distillation should be the method of choice for analysis of methyl mercury in Penobscot sediments.
- Chemical methylation of inorganic mercury during distillation should continue to be monitored.
- For river and estuarine sediment samples taken in 2007, it was recommended that 20% of samples should be done by both extraction and distillation, for comparison to 2006 sediment data obtained used extraction. This need not apply to wetland samples, because none were taken in 2006. It should also not be necessary to do this in 2008.

V. Tissues

A large variety of biological tissues have been collected during Phase I of the Penobscot Mercury Study. Total mercury in tissues was measured after acid digestion, or thermal decomposition. Methyl mercury was measured after a potassium hydroxide digestion (see Appendix E for details of methods).

Analytical Precision--Analytical duplicates

Data for precision (relative percent difference) of analytical duplicates done on tissue samples were compiled for a randomly chosen subset of these tissue types (Table 19), which included *Nereis* (worms), bird eggs and blood, and a variety of mammalian tissues (blood, fur, liver, muscle and brain).

Table 19. Relative percent difference (RPD) for analytical duplicates of randomly selected tissue types.

Tissue	Laboratory	Analysis	Mean % RPD	n
Nereis 2006	Flett Research	THg	7.6 +/- 8.7	**17**
Nereis 2006	Flett Research	MeHg	9.1+/- 9.7	**37**
Bird eggs 2006	Battelle	THg	3.8 +/- 3.3	**5**
Bird blood 2006	Battelle	THg	5/5 +/- 4.0	**5**
Bird eggs 2007	Battelle	THg	2.8 +/- 2.2	**6**
Bird blood 2007	Battelle	THg	5.7 +/- 3.9	**3**
Mammal tissues 2006	Battelle	THg	5.2 +/- 5.5	**15**
Mammal tissues 2006	**Battelle**	**THg**	**6.3 +/- 4.0**	**8**

The relative percent differences between analytical duplicates were all less than 10%. While there are no specific guidelines for this statistic, this degree of analytical agreement seems very adequate for the purpose of the data being collected. It is important that this statistic be compiled for each tissue type that is analyzed, and that it be compared to the variation among individual samples, and among years and sites, i.e., any differences seen that are less than the analytical RPD would not be considered as real differences among samples.

Interlab Comparison—Tissues.

Tissue samples were sent to the three participating laboratories in May 2007. In 2007, two laboratories reported results for total mercury in tissues (Figure 12), and three laboratories reported results for methyl mercury (Figure 13). In 2008, three laboratories reported results for both total mercury and methyl mercury (Figures 14 and 15). Tissue types were *Mytilus edulis* (blue mussel), *Nereis* (worm), *Mya arenaria* (soft-shelled crab) *Carcinus maenas* (common shore crab), scallops, lobster and fish.

All tissue results, for both total mercury and methyl mercury, were obviously in very good agreement in both years (Figures 12-15).

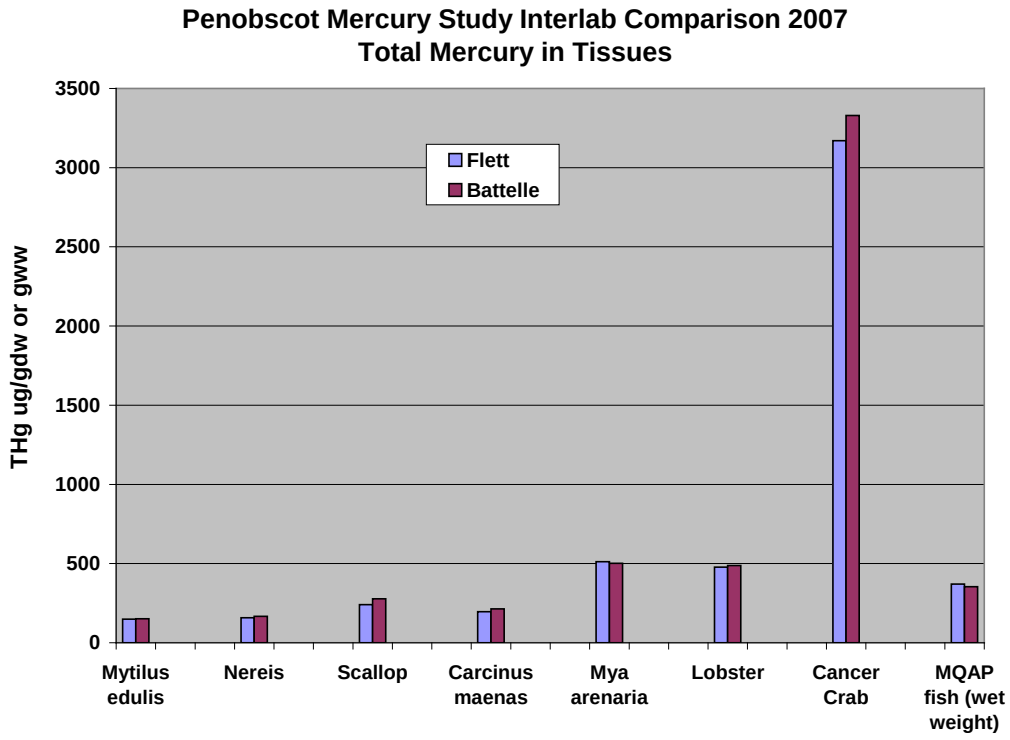


Figure 12. Total mercury in tissue, in interlab comparison tissue samples. Samples were collected in September and October, 2006, prepared as dry powders at Flett Research Ltd, except for the fish sample, which was sent as wet tissue. Samples were sent to laboratories in May 2007.

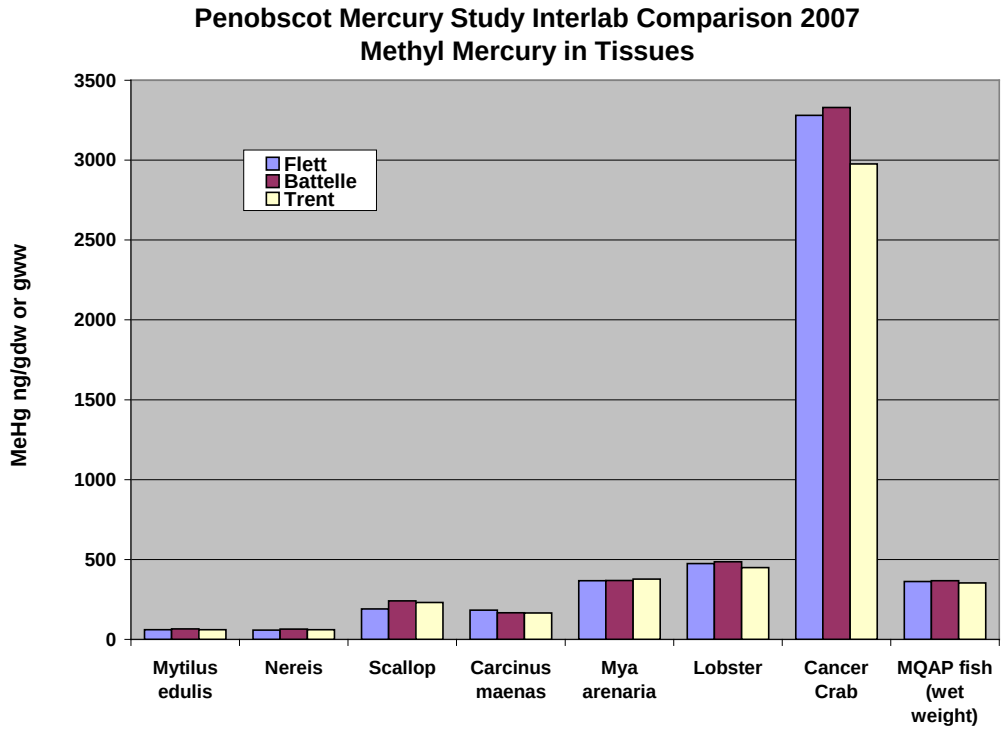


Figure 13. Methyl mercury concentrations in tissues, interlab comparison. Samples were collected in September and October, 2006, prepared as dry powders at Flett Research Ltd, except for the fish sample, which was sent as wet tissue. Samples were sent to laboratories in May 2007.

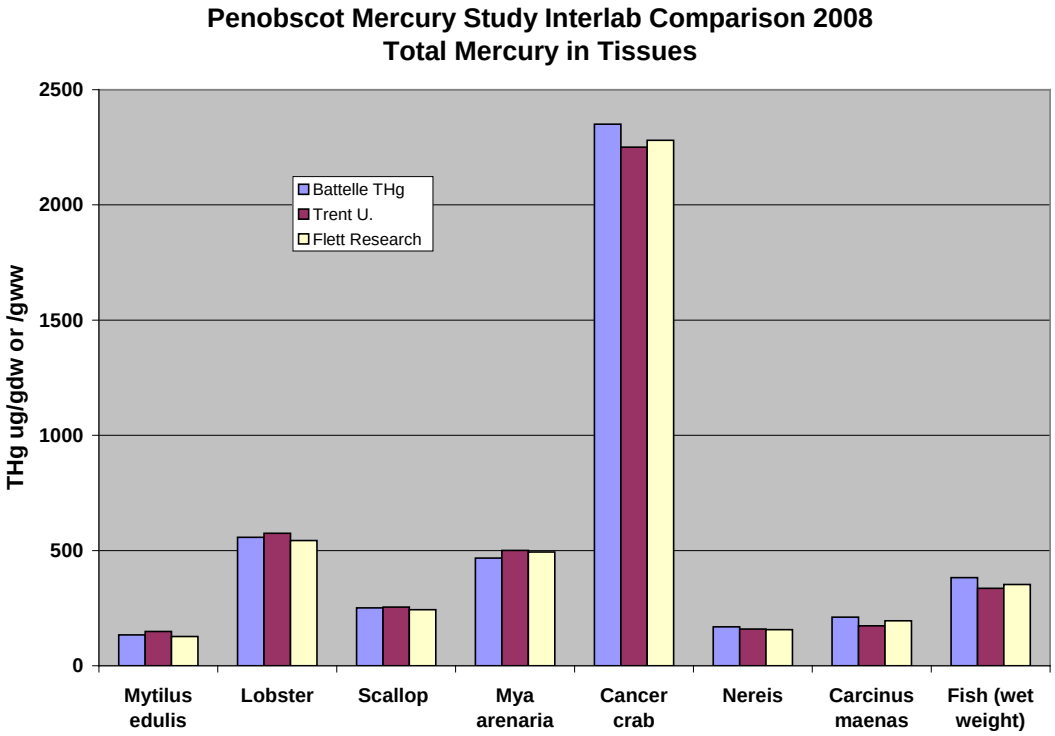


Figure 14. Total mercury in tissue, in interlab comparison tissue samples. Samples were collected in 2008 and prepared as dry powders at Flett Research Ltd, except for the fish sample, which was sent as wet tissue. Samples were sent to laboratories in June, 2008.

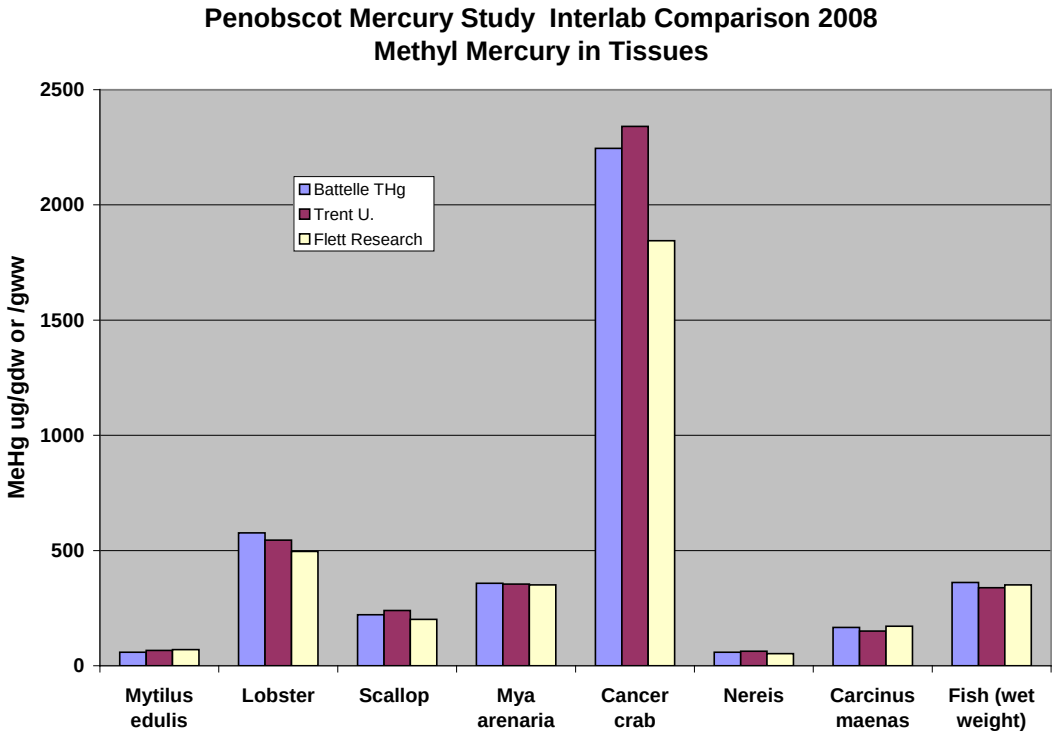


Figure 15. Methyl mercury in tissue, in interlab comparison tissue samples. Samples were collected in 2008 and prepared as dry powders at Flett Research Ltd, except for the fish sample, which was sent as wet tissue. Samples were sent to laboratories in June, 2008.

Recommendations with respect to tissue samples

The duplicate data on analysis of tissue samples, and the interlab comparison, indicated that there were no problems in this area. The interlab comparisons in both 2007 and 2008 demonstrated excellent agreement. The only recommendation is that analytical duplicate data should be compiled for each tissue type, as part of overall data analysis of samples.

References

Hintelmann, H., R. Falter, G. Hgen, R.D. Evans. 1997. Determination of artifactual formation of monomethylmercury (CH3Hg+) in environmental samples using stable Hg2+ isotopes with ICP-MS detection" Calculation of contents applying species specific isotope addition. Fresenius J. Anal. Chem 358: 363-370.

Horvat, M., N.S. Bloom, L. Liang. 1993. Comparison of distillation with other current isolation methods for the determination of methyl mercury compounds in low level environmental samples. Analytica Chimica Acta 281: 135-152.

Method 1631, Revision E. Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry. August 2002. U.S. Environmental Protection Agency. 35 pp.

EPA Method 1630. Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry. August, 1998. U.S. Environmental Protection Agency, Washington, D.C. 41 pp.

EPA Method 7473. Mercury in solids and Solutions by Thermal Decomposition , Amalgamation, and Atomic Absorption Spectrophotometry. February 2007. U.S. Environmental Protection Agency, Washington, D.C. 17 pp.

Appendix A. Summary of methods used by the laboratories.

Sample Type and analysis	**Battelle Marine Science Laboratory**	**Flett Research Ltd.**	**Trent U.**
Total Hg in water	EPA 1631e, oxidation and reduction, purge and trap, Cold Vapor Atomic Fluorescence Spectropscopy (CVAFS)	EPA 1631e, oxidation and reduction, purge and trap, Cold Vapor Atomic Fluorescence Spectropscopy (CVAFS)	**Oxidation and reduction, Isotope Dilution Mass Spectroscopy (IDMS)**
MeHg in water	EPA 1630, distillation, ethylation, purge and trap, CVAFS	EPA 1630, distillation, ethylation, purge and trap, CVAFS	**distillation, ethylation, purge and trap, IDMS**
Total Hg in sediments	EPA 7473, Thermal decomposition, CVAS	EPA 1631e, Digestion, purge and trap, CVAFS	**Acid digestion, $SnCl_2$ reduction, cold vapor flow, IDMS**
MeHg in sediments	Extraction and ethylation or distillation and ethylation, purge and trap, CVAFS, (adaptation of EPA 1630)	Extraction and ethylation or distillation and ethylation, purge and trap, CVAFS, (adaptation of EPA 1630)	**Distillation, ethylation, purge and trap, IDMS**
Total Hg in tissues	EPA 7473 CVAA or EPA 1631e CVAF	EPA 7473 CVAA with Direct Mercury Analyzer (DMA-80) or EPA 1631e after acid digestion	**Acid digestion, $SNCl_2$ reduction, cold vapor flow, IDMS**
MeHg in tissues	**KOH digestion, followed by EPA 1630 (ethylation, purge and trap, CVAFS)**	**KOH digestion, followed by EPA 1630 (ethylation, purge and trap, CVAFS)**	**KOH digestion, ethylation, purge and trap, IDMS**

Appendix B. Summary of reference materials used in the analyses of samples.

Sample Type and analysis	Battelle Marine Science Laboratory	Flett Research Ltd.	Trent U.
Total Hg in water	NIST 1641d	Baker QCS	**ORMS-3**
MeHg in water (no certified reference material available)	Laboratory preparation (DORM-2, diluted)	Laboratory preparation; Alfa standard (purchased)	**Laboratory preparation**
Total Hg in sediments	IAEA-405	MESS-2, NRC	**MESS-3**
MeHg in sediments	IAEA-405	IAEA-405	**IAEA-405**
Total Hg in tissues	DOLT-2	DORM-2 or DORM-3	**DORM-3**
MeHg in tissues	**DOLT-2**	**DORM-3**	**TORT-2, DORM-3**

NIST = National Institute of Standards and Technology (U.S.)
DORM = dogfish muscle, National Research Council (NRC), Canada
DOLT = dogfish liver, NRC, Canada
IAEA = International Atomic Energy Agency
TORT = lobster hepatopancreas, NRC, Canada
MESS = Marine sediment, Beaufort Sea, NRC, Canada
ORMS = Ottawa River water spiked with mercury, NRC, Canada

Appendix C. Details of Water analyses methods used by Battelle Marine Sciences Laboratory and Flett Research Ltd.

Total Mercury in water: Both laboratories used variations of EPA Method 1631e "Mercury in water by oxidation, purge and trap, and Cold Vapor Atomic Fluorescence Spectrometry (CVFAS)". In this method, all mercury is first oxidized to Hg(II), and then reduced to Hg^0 by addition of stannous chloride ($SnCl_2$). The gaseous mercury is then purged with gas, and trapped onto gold-coated sand traps. This Hg is then thermally desorbed onto a second trap and desorbed again, or may be directly desorbed into the analytical (fluorescence) cell for quantification.

Battelle Marine Sciences Laboratory.
Analytical Method: All samples stipulated for total mercury were analyzed by EPA Method 1631e.
Detection Limit: For total mercury in water, the achieved detection limit was 0.188 ng THg/L.
Quality Assurance Material: The standard reference material used for total mercury in water was NIST 1641d (1590000 +/- 1800 ng THg/L).
Recovery, accuracy and precision objectives: The accuracy of results for the standard reference material, and of ongoing precision sample, must be within +/- 23% of the expected value. The range of recovery of mercury standard added to samples (spike matrix recoveries) must be within 71-125%. The relative percent difference (RPD) for duplicate analyses of the same sample must be $\leq$ 21%.

Flett Research Ltd.
Analytical Method: EPA 1631e, Total Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectroscopy (CVAFS) (FR internal method #T00120, version 3).
Detection Limit: Minimum detection limit **(**MDL) = 0.04 ng Hg/L (based on 7 replicates of analytical blanks (99% confidence level)). The practical method limit (ML) of 0.5 ng/L, as stated in Method 1631e, has been adopted for our laboratory to reflect occasional elevated bottle blanks (< 0.5 ng/L) observed in reused acid-cleaned Teflon bottles filled with DI water.
Quality assurance material: OPR (Ongoing Precision Reference) solutions, which are large batches of water made up with a THg concentration within the range of usual samples. Each batch is large enough to provide a reference sample that is run on multiple consecutive dates, to check for day to day variance in analytical results, and variance within one day. Recovery must be within 77-133% of the expected value, and it is done once per every 10 samples. A second reference solution is Baker Quality Control Solution (QCS), with a certified concentration of 1000 ng/L. Recovery on this solution must be within 85-115% of the expected value.
Matrix effects: Recovery of total mercury spikes added to samples should be within 71-125% of the expected value, with RPD between duplicates <24%. Spike Matrix additions are done at a rate of once for every 10 samples.
Precision: The relative percent difference (RPD) between duplicate analyses must be <24%.

Estimated uncertainty: The estimated uncertainty of this method has preliminarily been determined to be ± 14.7 % @ 95 % confidence at a concentration level of 0.2 - 50 ng/L.

Methyl mercury in water:

Battelle Marine Sciences Laboratory

Analytical Method: All samples stipulated for methylmercury were distilled by the method of Horvat, et al., 1993 for methyl mercury. Samples were analyzed for methyl mercury by EPA Method 1630. Methylmercury in the distilled sample was ethylated and then purged onto carbon traps as a means of preconcentration and interference removal. The ethylated methylmercury was thermally desorbed into a fluorescence cell. Fluorescence (peak area) is proportional to the quantity of methylmercury collected, which is quantified using an average response factor as a function of the quantity of sample purged.
Detection Limit: For methyl mercury in water, the achieved detection limit was 0.0188 ng MeHg/L.
Quality Assurance Material: There is no certified reference material for methyl mercury in water. Battelle routinely uses DORM-2 tissue, diluted to a suitable concentration.
Recovery, accuracy and precision objectives: The accuracy of measurement of standard reference materials, or of ongoing precision samples, must be within +/- 33% of the expected value. The relative precision (relative percent difference) of analytical duplicates must be ≤ 35%. The range of recovery of methyl mercury standard added to samples (spike matrix recovery) must be with 65-135% of the expected concentration.

Flett Research Ltd.

Analytical Method: EPA (proposed) Method 1630; FR internal method # M10110 (Version 3): Methyl Mercury in water by distillation, ethylation, purge and trap, and CVAFS.
Detection Limit: MDL = 0.048 ng/L; ML = 0.14 ng/L. The method detection limit (MDL) is calculated to be the concentration equivalent to approximately three times the standard deviation of replicate measurements of the analyte in the given matrix at a concentration at or near the detection limit. (99% confidence level, 6 degrees of freedom). Client results are flagged below the ML.
Estimated Uncertainty: The estimated uncertainty of this method has preliminarily been determined to be ± 22 % at 0.5 ng/L (95 % confidence).
Quality assurance material: There is no certified reference material for methyl mercury in water. On each day when analyses are done, two reference materials are analyzed. The ongoing precision reference (MeOPR, 1000ng/L) is a 1/100 dilution of a lab standard "Y" solution, which was originally prepared in this lab from solid MeHgCl dissolved in isopropanol, diluted, and preserved with 0.05% acetic acid and 0.2% HCl. Recoveries must be with 77-123% of the expected value. The second material (Alfa, 200 ng MeHg/L) is a purchased standard. Mean recovery must be with 80-111% of the expected value.
Matrix effects: Recovery of methyl mercury spikes added to samples should be within 71-125% of the expected value, with RPD between duplicate spikes <24%. . Spike Matrix additions are done at a rate of once for every 10 samples.
Precision: The relative percent difference (RPD) between duplicate analyses must be <20%.

Appendix D. Analytical methods for mercury and methyl mercury in sediments used by Battelle Marine Sciences Laboratory and Flett Research Ltd.

Mercury in Sediments:

Battelle Marine Sciences Laboratory.
Analytical Method: All samples stipulated for total mercury were analyzed for total mercury by EPA Method 7473 (Thermal Decomposition, Amalgamation, and Cold Vapor Atomic Spectrophotometry). Samples were analyzed within the EPA holding time of 180 days.
Detection Limit: 3.2 ng/g
Quality Assurance material: The standard reference material (SRM) was IAEA-405. The criteria for recovery of total Hg from the SRM was 80-120%.
Precision: The relative percent difference between analytical duplicates must be $\leq$ 25%.
Matrix effects: Known quantities of total mercury are added to selected samples, and the recovery of this spike is quantified. Recoveries must be with +/- 20% of the expected value.

Flett Research Ltd.
Analytical Method: Total Mercury in Sediment, Soil, and Peat by Digestion, Purge and Trap, and CVAFS, adaptation of EPA Method 1631e, FR internal method # T00130, version 3.
Detection Limit: e.g. MDL = 2.4ng/g The method detection limit (MDL) is calculated to be the concentration equivalent to approximately three times the standard deviation of replicate measurements of the analyte in method blanks. (99% confidence level, 6 degrees of freedom) This limit assumes a 100 mg sample size. Lower detection limits are possible if greater sample weights are used.
Estimated Uncertainty: Preliminary determination: ± 18 % @ 95 % confidence at a concentration level of 40-100 ng/g; ± 32 % @ 95 % confidence at a concentration level of < 15 ng/g.
Reference Material: On each day when analyses are done, a certified reference material (Mess-2, 92ng Hg/g, from the National Research Council) is analyzed and compared to the certified concentration (the expected concentration). The total mercury concentration obtained must be within 80-110% of the expected value.
Precision: The relative percent difference (RPD) between duplicate analyses must be <20%.
Matrix effects: Known quantities of total mercury are added to selected samples. The recovery of this added mercury must be 71-125% of the expected value.

Methyl Mercury in Sediments:

Battelle Marine Sciences Laboratory
Methyl Mercury in Sediment: In 2006, all samples stipulated for methylmercury were extracted by the method of Bloom et al, 1997 for methyl mercury. Samples were analyzed by a modification of EPA Method 1630. Methylmercury in the extracted sample was ethylated and then purged onto carbon traps as a means of pre-concentration and interference removal. The ethylated methylmercury was thermally desorbed into a fluorescence cell. Fluorescence (peak area) is proportional to the quantity of methylmercury collected, which is quantified using an average response factor as a function of the quantity of sample purged. Samples were analyzed within the EPA holding time of 180 days. In 2007, Battelle began

also using the distillation method for methylmercury in sediments. In this method, sulfuric acid and KCl are added to thawed sediments, and distillation is carried out at 125 C in a Teflon still. The distillate is ethylated, and the ethyl methyl mercury is collected by purge and trap.
Detection Limit (both methods): 0.016-0.017 ng MeHg/gdw.
Reference Material (both methods): The standard reference material was IAEA-405. The criteria for recovery was 65-135%.
Precision: The relative percent difference (RPD) between analytical duplicates must be ≤ 35%.
Matrix effects: Known quantities of methyl mercury are added to selected samples, and the recovery of this methyl mercury should be with 65-135% of the expected values.

Flett Research
Analytical Method: Methyl Mercury in sediment by distillation, ethylation, purge and trap, and CVAFS, adaptation of EPA Method 1630, FR internal method #M10140, Version 3. Sulfuric acid and KCl are added to thawed sediment, and distillation is carried out at 147 C in a Teflon still. The distillate is ethylated, and the ethyl methyl mercury is collected by purge and trap.
Detection Limit: MDL = 0.02 ng/g based on 7 replicates of analytical blanks (99% confidence level).
Estimated Uncertainty: ± 18.3 % @ 95 % confidence at a concentration level of 0.1-50 ng/g
Reference material: On each day when analyses are done, a certified reference material (IAEA 405, 5.49 ng MeHg/g ±0.53, from the International Atomic Energy Agency) is analyzed and compared to the certified concentration (the expected concentration). The values obtained should be within 67-133% of the expected value.
Precision: The relative percent difference between analytical duplicates should be < 30 %.
Matrix effects: Known quantities of methyl mercury are added to selected samples, and the recovery of this methyl mercury should be with 65-135% of the expected values.

Appendix E. Analytical methods for mercury and methyl mercury in tissues used by Battelle Marine Sciences Laboratory and Flett Research Ltd.

Total Mercury in tissues:

Flett Research Ltd.
Analytical Method: EPA Method 7473 CVAA, using automated DMA-80, and EPA method 1631e after a nitric/sulfuric acid digestion of the tissue.
Detection Limit: MDL = 2.0 ng/g based on sets of 7 replicates of analytical blanks (99% confidence level, 6 degrees of freedom). This limit assumes a 200 mg wet sample size. Lower detection limits are possible if greater sample weights are used.
Estimated Uncertainty: At the 95 % confidence level, uncertainty has been preliminarily estimated at ± 12 % for fish muscle, 17.3 % for liver tissue, 27.8 % for fatty tissue, and 20.7 % for plant tissue.
Precision: The relative per cent difference between duplicate analyses must be less than 20%.
Reference Material: On each day when analyses are done, a certified reference material (DORM-2, 4640 ng/g, or DORM-3, 409 ng/g) is analyzed and compared to the certified concentration (the expected concentration). The mean recovery result must be within 80-110% of the expected concentration.
Recovery efficiency: In addition to determining the recovery efficiency of a certified reference material, known additions of Hg are made to selected samples, and the recovery efficiencies of these additions ("spike matrix additions") are determined. These must be within an acceptable limit (71-125% of expected value).
Correction of sample results for recovery efficiency: The recovery efficiency of the standard reference material is used to adjust the sample results for this factor.

Battelle Marine Sciences Laboratory
Analytical Method: Total mercury in liver and muscle was analyzed by EPA Method 7473 CVAA. Total mercury in blood and feathers was analyzed by EPA Method 1631e CVAF.
Achieved Detection Limit: 3.07 ngTHg/g
Reference Material: DOLT-2, certified value = 2.14 +/- 0.28 ugTHg/gdw. One sample of this material is analyzed with each batch of 20 samples, or each day if fewer than 20 samples are run. The analytical results should be within +/- 20% of the certified value.
Precision: One to two samples are analyzed in duplicate with each batch of 20 samples. The relative percent difference between replicate samples should be within 25%.
Spike Matrix Recoveries: Two matrix spike duplicate pairs are done with each batch of 20 samples. Recovery of known spikes of total mercury to sample matrices should be within 80 to 120% of the added mercury.

Methyl Mercury in tissues:

Flett Research Ltd.
Analytical Method: M10120: Methyl Mercury in biological tissue by KOH digestion, ethylation, purge and trap, and CVAFS (Version 3). From the digestion step forward, the method is similar to methyl mercury in water (EPA 1630).
Detection Limit: MDL = 0.11 ng/g. The MDL was determined based on 7 replicates of analytical blanks (99% confidence level).
Estimated Uncertainty: The estimated uncertainty (95% CI) of this method has preliminarily been determined to be + 68% at a concentration level of 0.1 ng/g and 22.4% at a concentration level of 4470 ng/g, based on 7 measurements.
Precision: The relative percent difference (RPD) between analytical duplicates should be less than 30%.
Reference Material: On each day when analyses are done, a certified reference material (DORM-2, 4640 ng/g) is analyzed and compared to the certified concentration (the expected concentration). The analytical result should be within 78-113% of the expected reference value.
Recovery efficiency: In addition to determining the recovery efficiency of a certified reference material, known additions of MeHg are made to selected samples, and the recovery efficiencies of these additions are determined. The recovery efficiency should be with 65-135% of the expected value.
Correction of sample results for recovery efficiency: The recovery efficiency of the standard reference material is used to adjust the sample results for this factor.

Battelle Marine Sciences Laboratory
Analytical Method: Digestion in 25% KOH in methanol (liver and muscle) followed by a modification of EPA method 1630.
Achieved Detection Limit: 1.03-1.17 ng/g.
Precision: One to two samples are analyzed in duplicate with each batch of 20 samples. The relative percent difference (RPD) between duplicate analyses should be les than 35%.
Reference Material: DOLT-2, certified value = 0.693 +/- 0.053 ug MeHg/gdw. The analytical results should be within +/- 35% of this value.
Recovery efficiency: Two spike matrix duplicate pairs are done with each batch of 20 samples. The recovery of known spikes of reference material to samples (spike matrix recoveries) should be within 65-135% of the expected value.

Penobscot River Mercury Study
Third Interlab Comparison, Mercury analyses in Water

Dr. C.A. Kelly, R&K Research Inc.
March 12, 2009

Objective: To carry out routine monitoring of the quality of results obtained for total mercury and methyl mercury in water by the two major analytical laboratories participating in the Penobscot Mercury Study. These are Battelle Marine Science Laboratories and Flett Research Ltd. A third laboratory, Trent University, also participated in order to provide additional results for inter-comparison. Interlab comparisons are essential for evaluation of analytical results on environmental samples such as mercury and methyl mercury in water, because it is not possible to have standards that have all the same chemical characteristics as natural water samples. Thus, there is no "known" value for the check samples used. Rather, the evaluation is done on how closely the laboratories agree with each other, and on how variable the results are.

Procedure: Filtered water samples were collected at two sites in the Penobscot River system, by Normandeau Associates personnel. These samples were sent to the three participating laboratories where they were analyzed for total mercury and methyl mercury concentrations. The results were reported to C. Kelly, who collated and examined them for standard measures of inter-comparison. The contact persons at each lab were Brenda LaSorsa (BMSL), Robert Flett (FR), and Dr. Holger Hintelmann (TU).

Results: Overall, the intercomparison showed good agreement among the three labs for both total mercury and methyl mercury in water. Two standard objective calculations were applied to the results: 1) an examination of reproducibility within each lab, done by calculating the relative per cent difference (RPD) between analytical duplicates from a single sample, and between replicate samples from the same site and 2) an examination of the reproducibility of results among the laboratories by calculating the average result for all three labs and the RPD between each lab's results and this overall average. Details are below.

Total Mercury. Within each laboratory, reproducibility of results on same water sample was excellent (data not shown), and reproducibility on replicate samples was also excellent, with RPD's all less then a few percent (Table 1).

Table 1. Analytical results for filtered water, total mercury. Samples were collected September 3, 2008. The site average was calculated from the results for two separate samples taken at each site.

Lab	Sampling Site	Site Average	RPD (within lab)
		ng THg/L	
BMSL	Veazie	1.84	2.2 %
	Marsh	1.47	2.4 %
FR	Veazie	1.68	1.2 %
	Marsh	1.34	1.5 %
TU	Veazie	1.61	0.6 %
	Marsh	1.31	0.8 %

The reproducibility of results among the labs was very good (Table 2, Figure 1).

Table 2. Average results from each lab, and the average for all labs, for total mercury in filtered water. The RPD's (relative percent difference) were calculated as the difference between each lab's average result and the grand average of results from all 3 labs.

		BMSL		FR		TU	
Site	**Average, all labs**	**Lab Average**	**RPD**	**Lab Average**	**RPD**	**Lab Average**	**RPD**
	ng THg/L	**ng THg/L**		**ng THg/L**		**ng THg/L**	
Veazie	1.71	1.84	7.7%	1.68	1.7%	1.61	6.0%
Marsh	1.37	1.47	7.3%	1.34	2.4%	1.31	4.9%

Methyl Mercury. The reproducibility within each lab was within acceptable limits (RPD's < 35%).

Table 3. Analytical results for methyl mercury in water (filtered). The site average from each lab is calculated from the results for two replicate samples taken at each site.

Laboratory	Sampling site	Site Average	RPD (within lab)
		ng MeHg/L	
BMSL	Veazie	0.15	0.0%
	Marsh	0.04	9.2%
FR	Veazie	0.15	6.9%
	Marsh	0.01	28.6%
TU	Veazie	0.14	3.0%
	Marsh	0.04	2.7%

The reproducibility among the three laboratories was excellent for the Veazie samples, but poor for the Marsh sample (Table 4, Figure 2). The Marsh sample was not a very useful one, however, as the MeHg concentration was close to detection level and therefore inherently difficult to measure. The Veazie sample concentrations were well above detection, and results compared well, and so this site provided the only easily interpretable results.

Table 4. Relative percent differences (RPD's) for each lab compared to the 3 lab average.

		BMSL		FR		TU	
Site	**Overall Average**	**Site Average**	**% RPD**	**Site Average**	**% RPD**	**Site Average**	**% RPD**
	ng MeHg/L	**ng MeHg/L**		**ng MeHg/L**		**ng MeHg/L**	
Veazie	0.14	0.15	3.3%	0.145	1.9%	0.14	5.2%
Marsh	0.03	0.04	41.8%	0.012	62.5%	0.04	20.7%

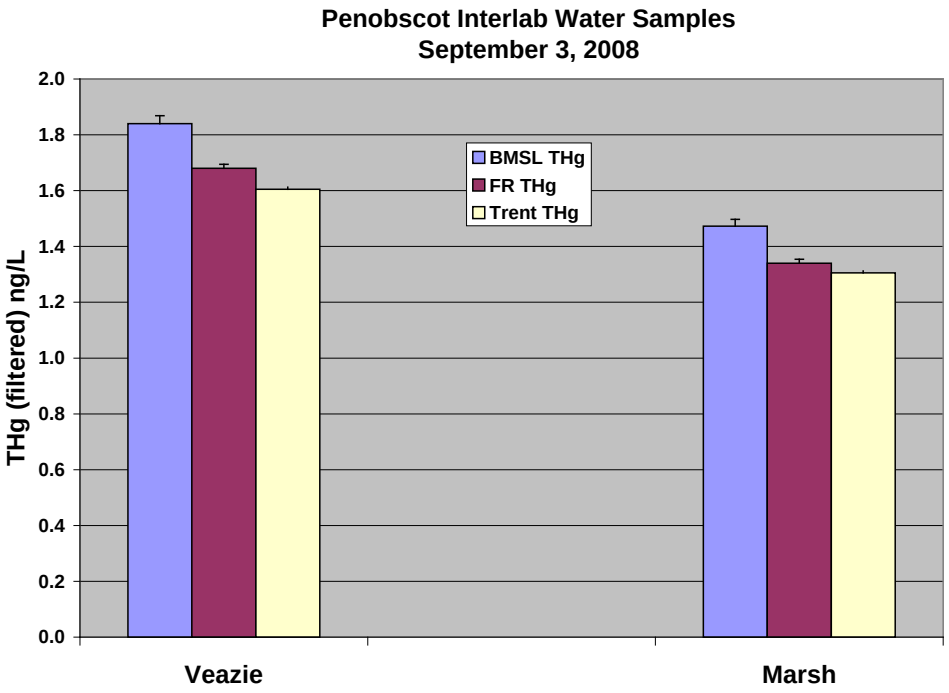


Figure 1. Average THg concentrations reported by each laboratory, for each sampling site. Bars are 1 standard deviation on the mean result from each laboratory.

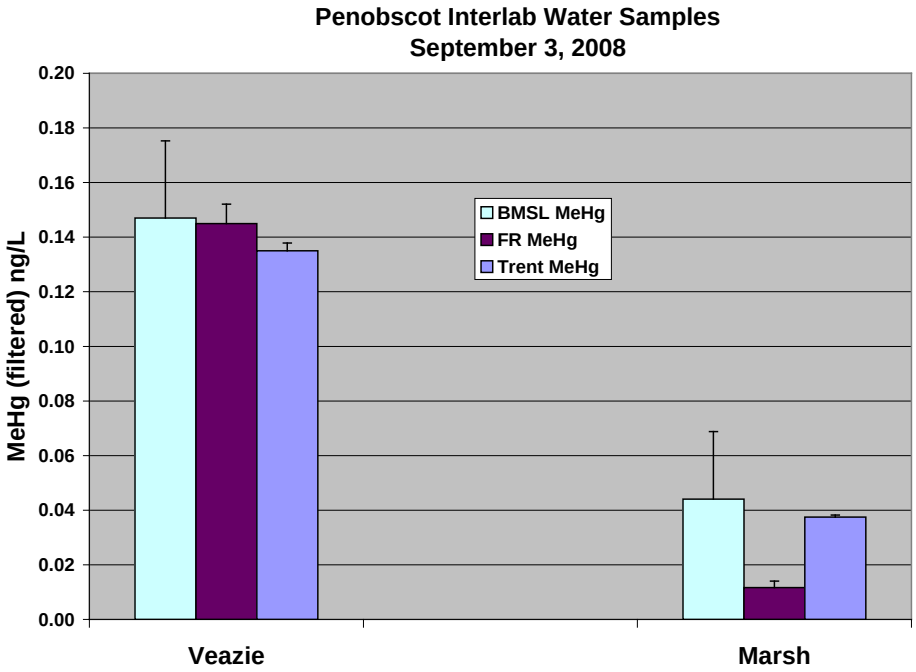


Figure 2. Average Methyl Mercury concentrations reported by each laboratory, for each sampling site. Bars are 1 standard deviation on the mean result from each laboratory.

Penobscot Mercury Study
Fourth Interlab Comparison, Mercury analyses in Water
Samples taken July, 2009

Dr. C.A. Kelly, R&K Research Inc.
December 18, 2009.

Objective: To carry out routine monitoring of the quality of results obtained for total mercury and methyl mercury in water by the major analytical laboratories participating in the Penobscot Mercury Study. These are Battelle Marine Sciences Laboratory in Sequim, WA, Flett Research Ltd. in Winnipeg, MB, and Dr. Cynthia Gilmore's laboratory at the Smithsonian Environmental Research Centre in MD. A fourth laboratory, Dr. Holger Hintelmann at Trent University in Peterborough, ON, also participated in order to provide additional results for inter-comparison. Interlab comparisons are essential for evaluation of analytical results on environmental samples such as mercury and methyl mercury in water, because it is not possible to have standards that have all the same chemical characteristics as natural water samples. Thus, there is no "known" value for the check samples used. Rather, the evaluation is done on how closely the laboratories agree with each other, and on how variable the results are.

Procedure: Water samples were collected at three sites, on July 7, 2009, in the Penobscot River system, by Normandeau Associates personnel. These samples were sent to the four participating laboratories where they were analyzed for total mercury and methyl mercury concentrations. The results were reported to C. Kelly, who collated and examined them for standard measures of inter-comparison.

Laboratory Methods: For total mercury in water, all labs used methods similar to EPA 1631e—bromine monochloride oxidation followed by stannous chloride reduction to elemental mercury. For methyl mercury in waater, all labs used methods similar to EPA 1630—distillation followed by ethylation. Quantification of mercury was by either cold vapor atomic fluorescence (CVAFS), or Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Results: Overall, the intercomparison showed very good agreement among the four labs for both total mercury (in unfiltered water) and methyl mercury (in filtered water). Two standard objective calculations were applied to the results: 1) an examination of reproducibility within each lab, done by calculating the relative per cent difference (RPD) between analytical duplicates from a single sample, and 2) an examination of the reproducibility of results among the laboratories by calculating the grand average result from all four labs, for each sample, and the RPD between each lab's results and this grand average. Details are below.

Analytical Duplicates. Within each of the three PRMS laboratories, reproducibility of results on same water sample was excellent, with RPD's all less then a few percent for total mercury, and less than 15% for methyl mercury (Table 1). All these results are well within the +/- 24% limit recommended by the EPA for duplicate reproducibility. (Trent U. did not provide analytical duplicate results.)

Table 1. Relative percent difference (RPD) between analytical duplicates for total mercury and methyl mercury in water.

	Total Mercury		Methyl Mercury	
	Average % RPD	n	Average % RPD	n
BMSL	2.16%	5	7.34%	5
Flett Research	1.25%	4	13.88%	4
SERC	3.96%	12	5.08%	9

Total Mercury in Water Results.

Results for total mercury in unfiltered water were obtained from four laboratories (Figure 1).

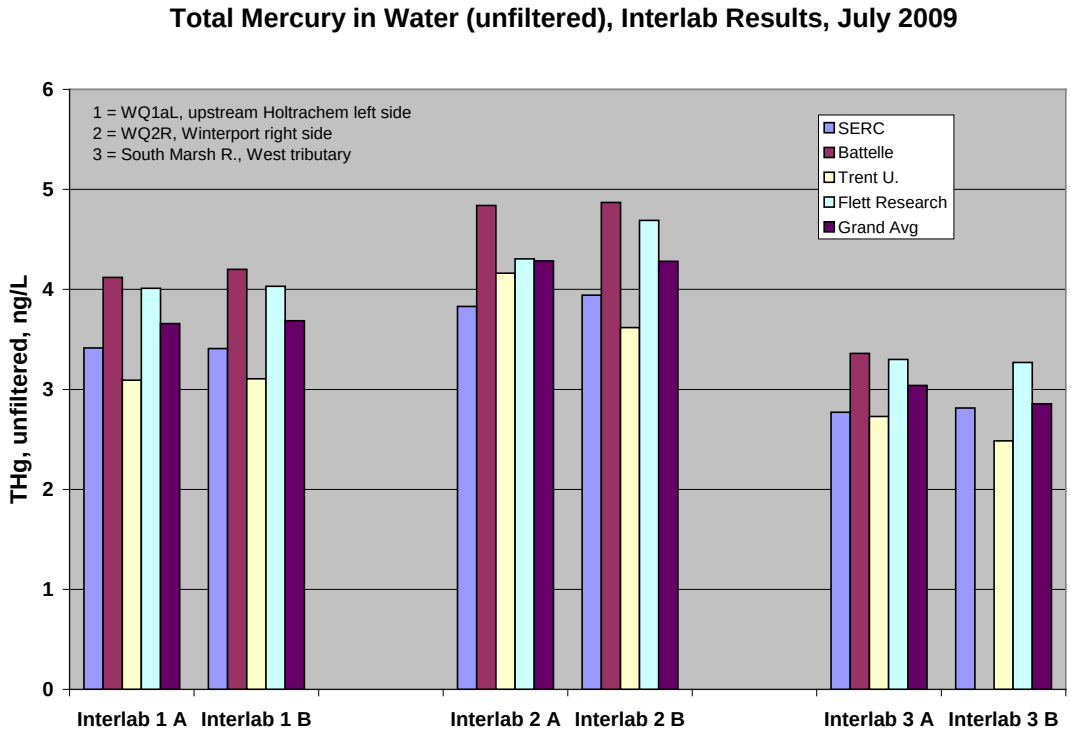


Figure 1. Total mercury concentrations measured by each laboratory in unfiltered water samples from the Penobscot River, and the grand average for each sample. Samples were collected July 7, 2009. A and B samples were two separate samples taken from the same site.

The standard approach to quantifying the reproducibility among the labs is to calculate the grand average of all lab results. This is considered the "correct" analytical result, since the sample is not a standard, and the actual value is unknown. Then each individual lab's result is compared to that average by calculating the RPD. These RPD's are shown in Table 2. All RPD's were within the recommended guideline for replicate samples (<20% for total mercury in water).

Table 2. Relative percent difference between the result of each laboratory and the grand average result for each sample.

		SERC	Battelle MSL	Trent U.	Flett Research
Sample	**Site**	**THg ng/L**	**THg ng/L**	**THg ng/L**	**THg ng/L**
		RPD	**RPD**	**RPD**	**RPD**
Interlab 1 A	WQ1aL	6.68%	12.60%	15.52%	9.59%
Interlab 1 B	WQ1aL	7.54%	13.95%	15.74%	9.33%
Interlab 2 A	WQ2R	10.60%	12.97%	2.86%	0.48%
Interlab 2 B	WQ2R	7.89%	13.79%	15.48%	9.58%
Interlab 3 A	Interlab 3	8.85%	10.52%	10.22%	8.55%
Interlab 3 B	Interlab 3	1.52%		12.98%	14.49%

Methyl Mercury in Water Results.

Filtered water samples from the same sites as above were taken and sent to each participating laboratory for analysis for methyl mercury. Results are shown in Figure 2.

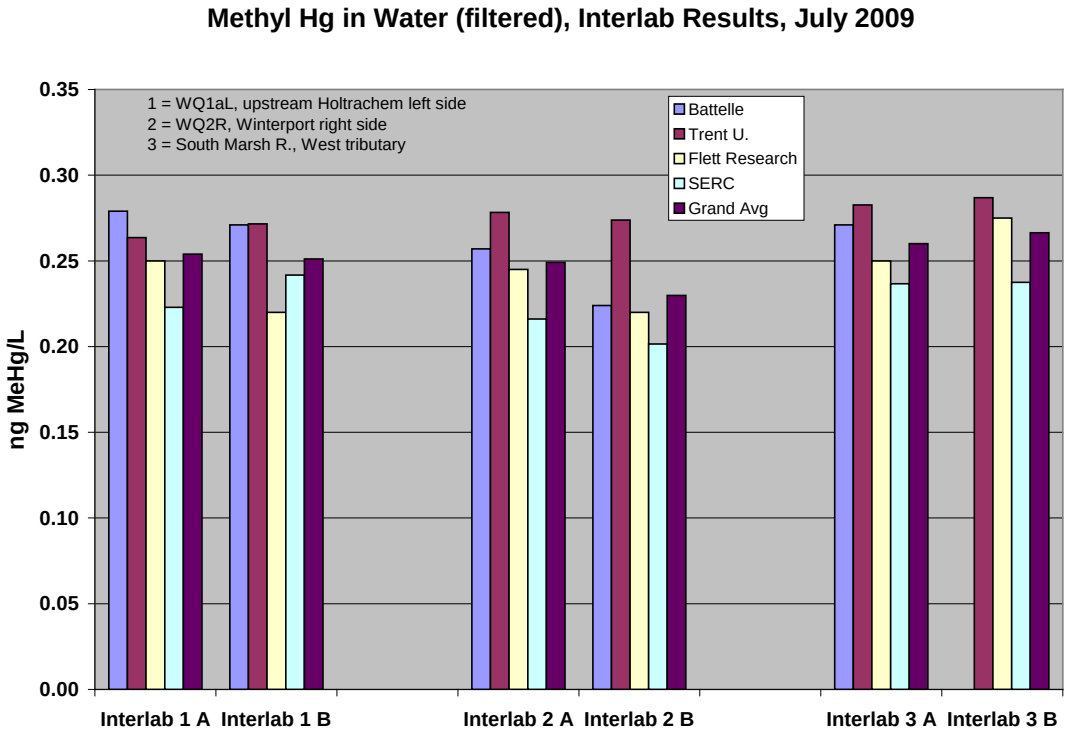


Figure 2. Analytical results for methyl mercury in water (filtered). The grand average is the average of results from all 4 laboratories for each sample.

The relative percent difference (RPD) between each sample result for methyl mercury, and the grand average, was calculated (Table 3). These RPD's were well within the recommended guideline for replicate samples of < 35% for methyl mercury in water.

Table 3. Relative percent differences (RPD's) for each lab compared to the 4 lab average, for methyl mercury in filtered water.

		SERC	Battelle	Trent U.	Flett Research
Sample	Site	MeHg	MeHg	MeHg	MeHg
		RPD	RPD	RPD	RPD
Interlab 1 A	WQ1aL	12.19%	9.89%	3.84%	1.53%
Interlab 1 B	WQ1aL	3.73%	7.94%	8.17%	12.38%
Interlab 2 A	WQ2R	13.26%	3.17%	11.74%	1.65%
Interlab 2 B	WQ2R	12.33%	2.53%	19.13%	4.27%
Interlab 3 A	Interlab 3	9.00%	4.21%	8.66%	3.87%
Interlab 3 B	Interlab 3	10.88%		7.67%	3.21%

	Total Hg in water	MeHg in water
Battelle Marine Science Laboratory	EPA 1631e, BrCl oxidation and $SnCl_2$ reduction, purge and trap on gold, Cold Vapor Atomic Fluorescence Spectropscopy (CVAFS)	EPA 1630, distillation, ethylation, purge and trap, CVAFS
Flett Research Ltd.	EPA 1631e, BrCl oxidation and $SnCl_2$ reduction, purge and trap, Cold Vapor Atomic Fluorescence Spectropscopy (CVAFS)	EPA 1630, distillation, ethylation, purge and trap, CVAFS
Smithsonian Environmental Research Center	EPA 1631e, Gill, Bloom BrCl oxidation, $SnCl_2$ reduction, purge and trap onto gold traps, heating and introduction directly into the ICP/MS using argon	EPA 1630 (Draft), Bloom, Horvat. Distillation, ethylation, purge and trap onto TENAX, heating onto Chromasorb, and introduction directly in the ICP/NS using argon
Trent U.	Isotope Dilution Mass Spectroscopy (IDMS). BrCl Oxidation and $SnCl_2$ reduction, direct purge into plasma of ICP/MS.	distillation, ethylation, purge and trap, IDMS

Report on Inter lab Comparison
Total and Methyl Mercury in Sediments

Penobscot River Mercury Study

June 15, 2010

Dr. Carol Kelly
R&K Research Inc.

One of the most important Quality Assurance/Quality control (QA/QC) goals of the Penobscot River Mercury Study is to carry out an interlab comparison, at least once a year, among the laboratories that perform mercury analyses in water, sediment and biota. A separate report is done for each of these three types of samples; this report covers the most recent interlab exercise for analyses of total mercury (THg) and methyl mercury (MeHg) in sediments.

Sediment grab samples were taken on August 31, 2009, at three sites in the Penobscot River system, by the Normandeau Associates field crew. At each site, a Van der Veen sampler was used to collect a sample. Sediment from the top 0-3 cm was collected into a large container, and mixed. A 125 mL polyethethylene container was filled for each laboratory. Sediment was frozen and shipped to each of the participating labs: Battelle Marine Sciences Laboratory (Sequim, WA), Flett Research Ltd. (Winnipeg, MB), Smithsonian Center for Ecosystem Research (Maryland), and Trent University (Peterborough, ON).

Methods. Brief summaries of the methods used in each laboratory are shown in Appendix A. More detailed descriptions are available.

Performance criteria. Because the "true" concentrations of THg and MeHg in the sediment samples collected are unknown, the performance criteria are based on two types of precision: individual sample precision within each laboratory, and precision among the different laboratories.

Precision of measurement within each laboratory is evaluated by examining results where duplicate subsamples (from one original sample) are analyzed. Precision is expressed as the Relative Percent Difference (RPD) between the two duplicates:

$$RPD = [(\text{Duplicate A} - \text{Duplicate B})/\text{Average of A \& B}]*100$$

The EPA criteria for THg in solids is RPD $\leq$ 30% (Appendix to Method 1631, EPA 2001). For MeHg, there is no "official" criterion. For the purposes of the PRMS, RPD $\leq$ 35% for MeHg in sediments is being used, based on acceptance criteria for analytical precision for MeHg in water (Method 1630, EPA 2001).

A similar approach is used for precision among the laboratories. In this case the above formula is modified so that the result from each laboratory is compared to the average result from all laboratories:

$$RPD_x = [(Lab_x \text{ result} - \text{Average result})/\text{Average result}] * 100$$

The numerical % criteria for THg and MeHg are the same as above.

Individual Sample Precision. Each sample from the three sites was analyzed in duplicate at least once for both THg and MeHg. Thus a minimum of 6 values for analytical precision were provided from each laboratory. Average RPD's for analytical duplicates in each laboratory are shown in Tables 1 and 2, for THg and MeHg respectively. No individual RPD was greater than the criterion.

Precision Among Laboratories. The measured concentrations obtained by each laboratory were compiled for each site, for THg (Figure 1) and for MeHg (Figure 2). The average THg concentration in sediments at each of the three different sites was calculated using data from all four laboratories (Figure 1). The average THg concentrations were 967 ng/gdw at W10 (Site 1), 1277 ng/gdw at W17 (Site 2) and 515 ng/gdw at W25 (Site 3). The average MeHg concentrations (Figure 2) were 21 ng/gdw at W10, 23 ng/gdw at W17, and 14 ng/gdw at W25. These average values were used as the assumed "correct" THg and MeHg value for each site.

The RPD's for THg analyses (Table 3) were all within the criterion for performance in EPA Method 1631 for Total Mercury (appendix for solids), which is RPD $\leq$30% for

replicate samples. These samples can be considered "replicates" because they were taken from a homogenized field sample.

For MeHg, one RPD was slightly higher than this (36%, Table 4), but overall, the RPD values were much lower (Table 4).

Overall recommendations: The results of this interlab comparison were satisfactory.

Table 1. Individual Sample Precision, measured as the relative percent differences (RPD's) for duplicate analyses performed within each laboratory, for total mercury in sediments. Acceptable values are RPD $\leq$ 30%.

Site	Flett Research Ltd	Battelle Marine Sciences Laboratory	Smithsonian Environmental Research Center	Trent U.
1 (W10)	5.10%	6.92%	4.69%	11.51%
			2.02%	11.91%
2 (W17)	4.15%	6.17%	2.88%	2.03%
			2.45%	2.23%
3 (W25)	7.90%	2.99%	1.24%	23.07%
			4.89%	17.74%

Table 2. Individual Sample Precision, measured as relative percent differences (RPD's) for duplicate analyses performed within each laboratory, for MeHg in sediments. Acceptable values are RPD $\leq$ 35%

Site	Flett Research Ltd	Battelle Marine Sciences Laboratory	Smithsonian Environmental Research Center	Trent U.
1 (W10)	4.33%	5.36%	3.31%	10.00%
	5.66%		3.00%	2.63%
2 (W17)	3.48%	6.30%	4.28%	5.14%
			3.49%	9.41%
3 (W25)	4.85%	3.13%	3.61%	12.43%
			0.00%	17.05%

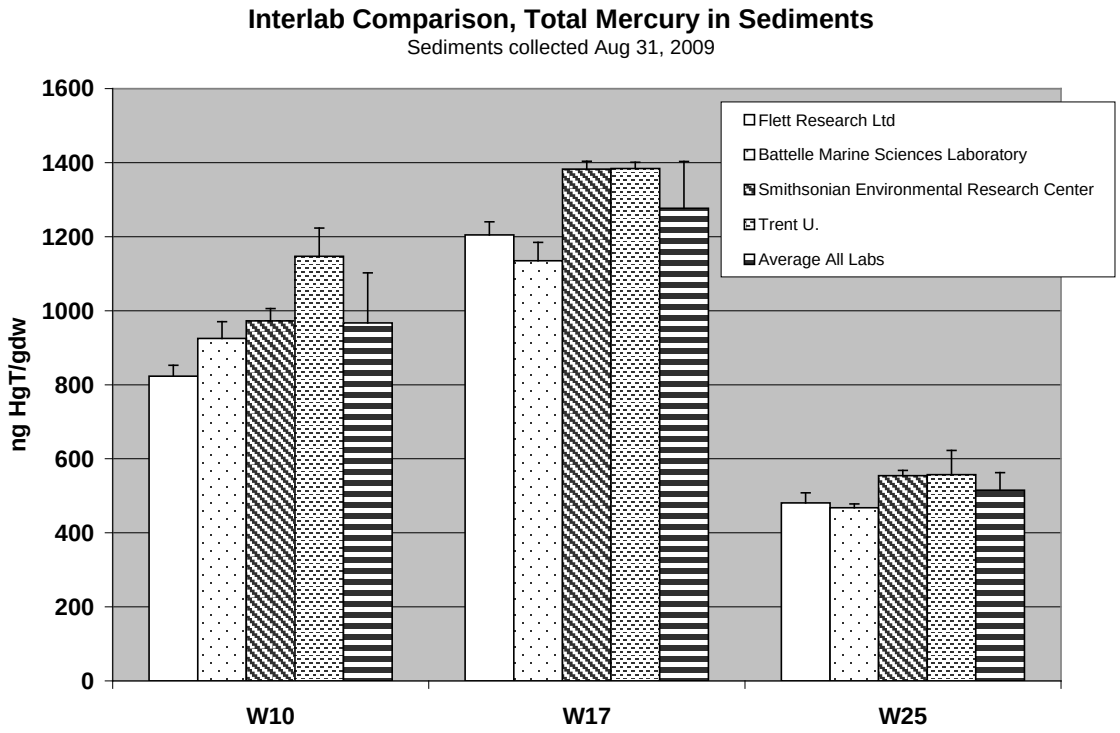


Figure 1. Concentrations of total mercury in sediment samples from 3 sites, measured by four different laboratories.

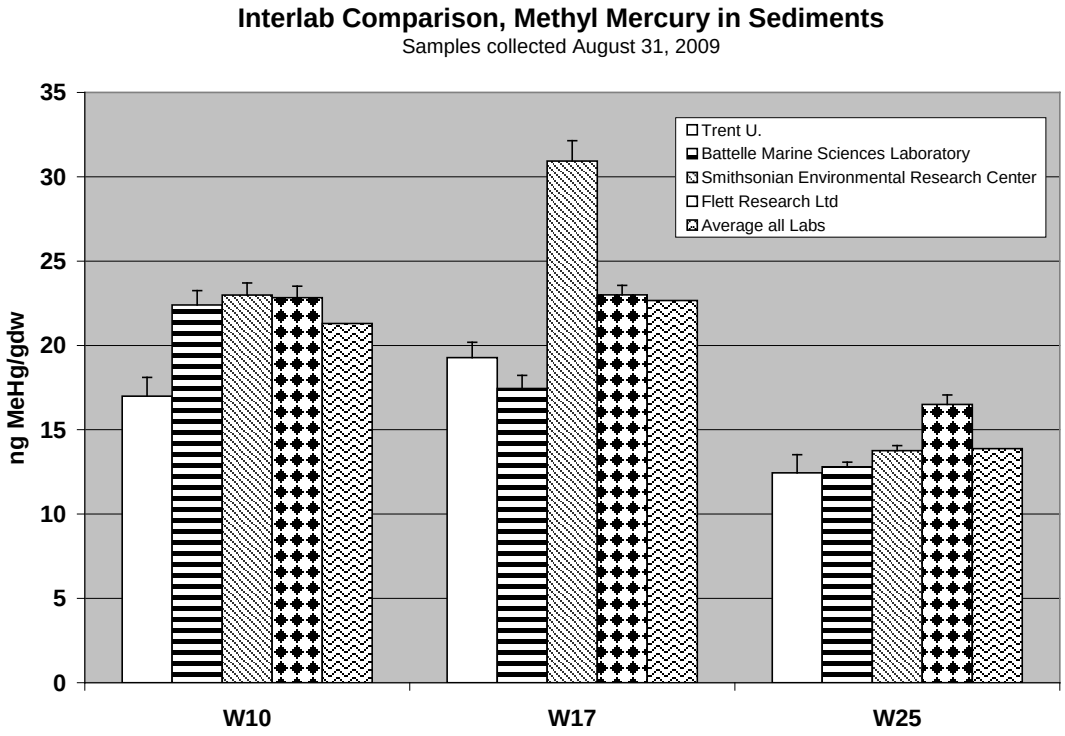


Figure 2. Concentrations of methyl mercury measured in sediments from three sites, by four different laboratories.

Table 3. Relative per cent differences (RPD's) between the result from each laboratory and the average result (of all four laboratories), for THg in sediment from each site.

	Site	Flett Research Ltd	Battelle Marine Sciences Laboratory	Smithsonian Environmental Research Center	Trent U.
1	W10	14.89%	4.34%	0.59%	18.64%
2	W17	5.61%	11.09%	8.28%	8.42%
3	W25	6.64%	9.16%	7.66%	8.13%

Table 4. Relative per cent differences (RPD's) between the result from each laboratory and the average result (of all four laboratories), for MeHg in sediment from each site.

Site		Flett Research Ltd	Battelle Marine Sciences Laboratory	Smithsonian Environmental Research Center	Trent U.
1	W10	7.19%	5.16%	7.89%	20.24%
2	W17	1.48%	23.01%	36.48%	14.95%
3	W25	18.90%	7.76%	0.80%	10.33%

Appendix A. Brief summaries of methods used in each laboratory.

Sample Type and analysis	Battelle Marine Science Laboratory	Flett Research Ltd.	Smithsonian Environmental Research Center	Trent U.
Total Hg in sediments	EPA 7473, Thermal decomposition, CVAFS	EPA 1631e, Acid digestion, BrCl oxidation, $SnCl_2$ reduction, purge and trap, CVAFS	Modification of EPA 1631, acid digestion, BrCl oxidation, $SnCl_2$ reduction, Flow Injection Automated Sampler (FIAS), ICP-MS	Acid digestion, BrCl oxidation, $SnCl_2$ reduction, cold vapor flow, isotope dilution and ICP-MS
THg Reference Material	IAEA-405	MESS-2, NRC	MESS-3, NRC	MESS-3, NRC
MeHg in sediments	Extraction or distillation, ethylation, purge and trap, CVAFS, (adaptation of EPA 1630)	Extraction or distillation, ethylation, purge and trap, CVAFS, (adaptation of EPA 1630)	Extraction or distillation, ethylation, purge and trap, isotope dilution and ICP-MS (adaptation of EPA 1630)	Distillation, ethylation, purge and trap, isotope dilution and ICP-MS
MeHg Reference Material	IAEA-405	IAEA-405	IAEA-405	IAEA-405

IAEA = International Atomic Energy Agency
MESS= Marine sediment, Beaufort Sea, National Research Council, Canada

Interlab Comparison,
Total and Methyl Mercury in Tissues

Penobscot River Mercury Study

August 17, 2010

C.A. Kelly
R&K Research Inc.

Three laboratories participated in an interlab comparison exercise, analyzing fish, *Mytilus*, and lobster tissues for total mercury (THg) and methyl mercury (MeHg). Battelle Marine Science Laboratories (BMSL) and Flett Research Ltd. routinely analyze samples for the Penobscot River Mercury Study (PRMS). The third laboratory (Dr. Holger Hintelman's lab at Trent University) participated at our request, in order to provide additional results for comparison. Dr. Hintelman is an internationally respected expert in the field of mercury analytical chemistry.

Tissues were sent by Dawn Gilbert (Flett Research) in September 2009. The mussel and lobster samples were dry tissue, while the fish sample was wet (canned). The canned fish was from the mercury quality assurance program of the Canadian Department of Fisheries and Oceans. It has been analyzed by 36 laboratories, with a mean total mercury concentration of 0.259 ± 0.039 mg THg/Kg, or 259 ng/gww.

This was the third interlab comparison on biota. The first two were carried out in 2007 and 2008, and were reported in the "Second Report on Interlab Comparisons and Quality Control/Quality Assurance Data from laboratories participating in the Penobscot Mercury Study, September 2008".

As in the first two biota interlab exercises for the PRMS, the results for both THg and MeHg concentrations, for all 3 tissue types, were in very good agreement (Figures 1, 2 and 3).

A standard approach to evaluating results of interlab comparisons is to take the grand average of results from all participating laboratories as the "correct" value for that tissue. Then the relative percent difference (RPD) between that value (V_{avg}) and the result from each individual laboratory (V_i) is calculated (Tables 1 and 2).

$$RPD = [(V_i - V_{avg})/((V_i + V_{avg})/2)]*100$$

The RPD's were all less than 7%. This is well within the recommended limits for RPD's ($\leq$ 30% for total mercury, EPA Method 1631 appendix for solids).

The average result for THg in fish was 258 ng/gww (Table 1), which was essentially the same as the result from 36 laboratories (259 ng/gww).

Overall, the results were very satisfactory. No new recommendations for tissue analyses are needed at this time.

Fig. 1. Total and Methyl Mercury in Lobster Tissue

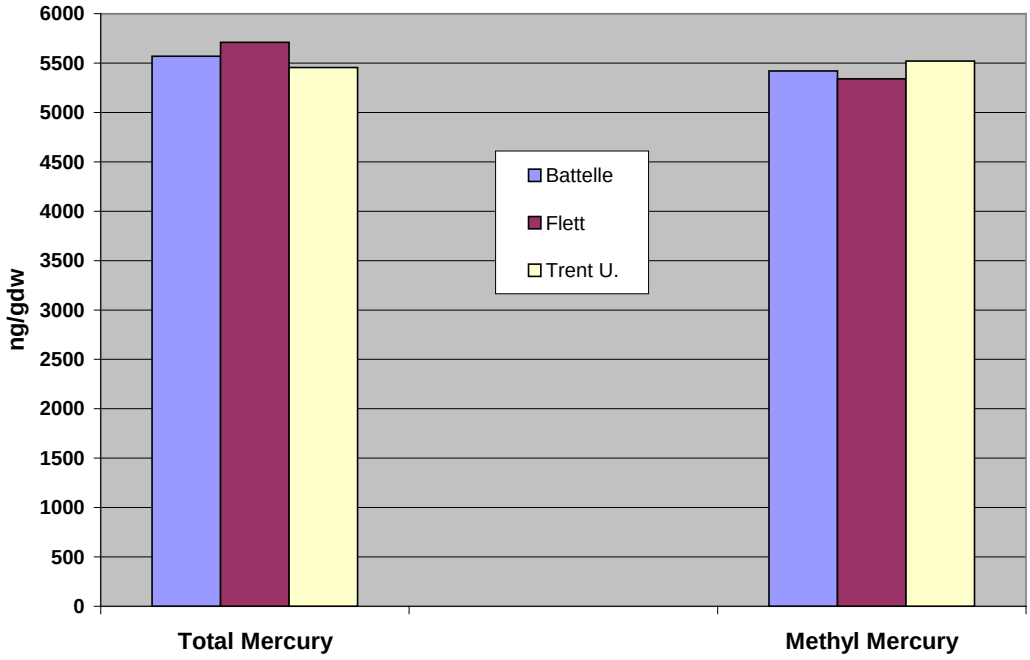


Fig. 2. Total and Methyl Mercury in Mytilus

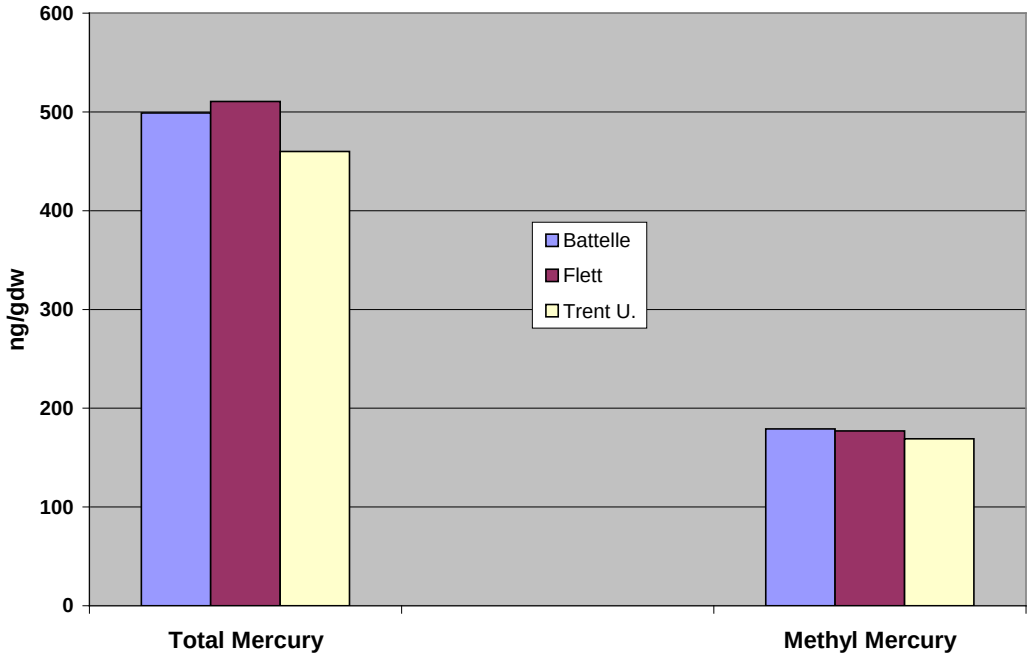


Fig. 3. Total and Methyl Mercury in Fish

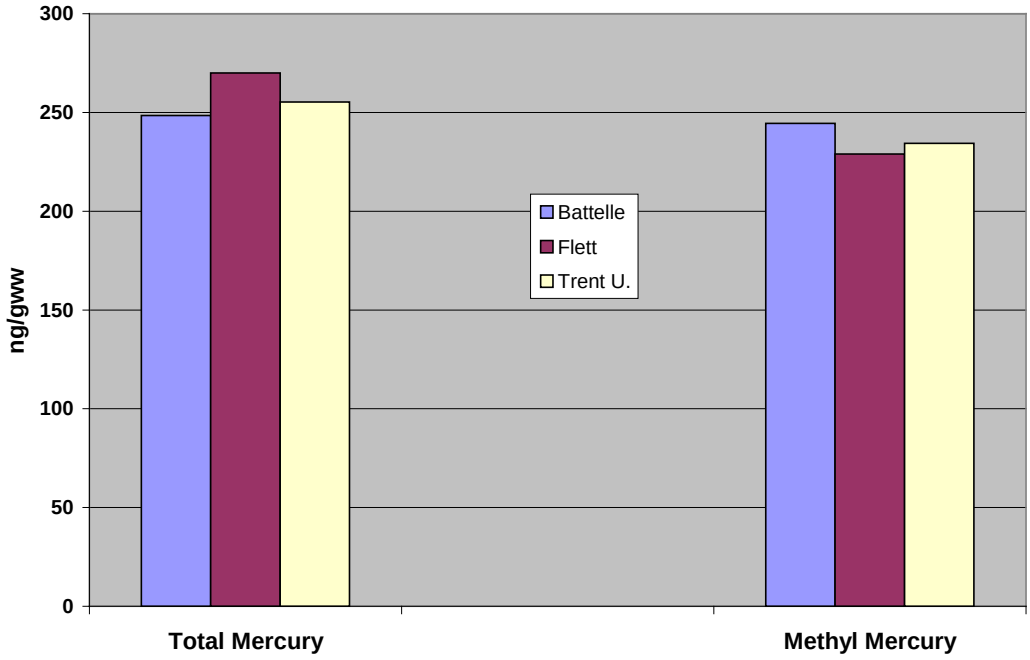


Table 1. Total Mercury concentrations measured in biota tissues.

	Average, all 3 labs	**BMSL**		**Flett Research**		**Trent U.**	
	ng/gdw or ng/gww*	ng/gdw or ng/gww*	RPD	ng/gdw or ng/gww*	RPD	ng/gdw or ng/gww*	RPD
Mytilus edulis (dry sample)	490	499	1.85%	511	4.13%	460	6.28%
Lobster Tail (dry sample)	5578	5570	0.15%	5710	2.33%	5455	2.24%
Fish (wet sample)	258	249	3.73%	270	4.57%	255	1.02%

Table 2. Methyl Mercury concentrations measured in biota tissues.

	Average, all 3 labs	**Battelle**		**Flett**		**Trent U.**	
	ng/gdw or ng/gww*	ng/gdw or ng/gww*	RPD	ng/gdw or ng/gww*	RPD	ng/gdw or ng/gww*	RPD
Mytilus edulis (dry sample)	175	179	2.26%	177	1.14%	169	3.49%
Lobster Tail (dry sample)	5427	5420	0.12%	5340	1.61%	5520	1.71%
Fish (wet sample)	236	245	3.56%	229	2.99%	234	0.69%

Penobscot River Mercury Study

Mercury in Water Quality Assurance: Fifth Inter-lab Comparison

Dr. C.A. Kelly, R&K Research Inc.
December 16, 2011

Objective:

To carry out routine monitoring of the quality of measurements of total mercury and methyl mercury concentrations in water samples taken for the Penobscot River Mercury Study (PRMS). Three aspects were examined: 1) precision of analytical duplicates, 2) precision of field replicates, and 3) interlab comparison of results from the major analytical laboratories participating in the Penobscot Mercury Study. Inter-lab comparisons are essential for evaluation of analyses of mercury and methyl mercury in natural water samples, because it is not possible to have standards that have all the same chemical characteristics, and potential interferences in analytical quality, as natural water samples. Thus, natural water samples are used and split, so that all participating laboratories analyze the samples. The evaluation is done on how variable the results are within each laboratory, and how closely the results from each laboratory agree with each other.

Procedure:

Water samples were collected at three sites in the Penobscot River system, by Normandeau Associates personnel, on July 28, 2010. At each site, both unfiltered and filered samples were collected, for a total of 6 sample types. Each of these sample types were taken in duplicate. These samples were sent to participating laboratories where they were analyzed for total mercury and methyl mercury concentrations. For this exercise, the three labs that analyzed samples in 2010 participated. These were Battelle Marine Sciences Laboratory in Sequim, WA, Flett Research Ltd. in Winnipeg, MB, and Dr. Cynthia Gilmore's laboratory at the Smithsonian Environmental Research Centre (SERC) in MD. Dr. Holger Hintelmann's laboratory at Trent University in Peterborough, ON also analyzed samples for MeHg. The results were reported to Dr. Carol Kelly, R&K Research Inc., who collated and examined them for standard measures of inter-comparison.

Laboratory Methods:

For total mercury in water, all labs used methods similar to EPA 1631e—bromine monochloride oxidation followed by stannous chloride reduction to elemental mercury. For methyl mercury in water, all labs used methods similar to EPA 1630—distillation followed by ethylation.

Quantification of mercury was by either cold vapor atomic fluorescence (CVAFS), or Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Results:

Overall, the intercomparison showed very good agreement among the four labs for both total mercury and methyl mercury in filtered and unfiltered water. Two standard objective calculations were applied to the results: 1) an examination of reproducibility within each lab, done by calculating the relative per cent difference (RPD) between analytical duplicates from a single sample, and 2) an examination of the reproducibility of results among the laboratories by calculating the grand average result from all three labs, for each sample, and the RPD between each lab's results and this grand average. Precision of field replicate samples was also examined, as a measure of variation that can occur when sampling more than once at the same site. This evaluation is not specifically a quality control measure of the laboratories, but is useful to the project in evaluating uncertainty involved in water sampling in the Penobscot system.

All analytical duplicate results were excellent, well within the ranges specified by EPA guidelines. The range of RPD's for analytical duplicates was 2.6-4.6% for THg and 4-11% for MeHg.

The RPD's for field replicates were greater than for analytical duplicates, as expected, and were 13-27% for THg, and 13-24% for MeHg.

Out of 18 inter comparisons of results for THg, (3 laboratories x 6 water samples), only 2 fell outside the EPA guideline of +/- 25%. Of the 24 inter-comparisons of results for MeHg (4 laboratories x 6 water samples), only 1 was outside the guideline of +/- 35%.

Details are below.

Total Mercury in Filtered and Unfiltered Water.

Analytical Duplicates. Each water sample was analyzed twice or three times for total mercury (THg). Within each of the three PRMS laboratories, reproducibility of results on the same water sample was very good. All duplicate RPD's were less than 15%, and most were less than 5% (Table 1). These RPD's were well within the +/- 24% limit recommended by the EPA for duplicate reproducibility using Method 1631 (USEPA, 2002). The average RPD between duplicates was 2.6 +/- 1.6% for Flett Research Ltd., 2.9 +/- 3.5 % for SERC, and 4.6 +/- 4.0 % for Battelle.

Table 1. Relative percent difference (RPD) between duplicate analyses for THg in water. "F" = filtered; "Uf" = unfiltered.

Water sample	Flett Duplicate RPD	SERC Duplicate RPD	SERC Duplicate RPD	Battelle Duplicate RPD
Interlab 1 Fa	0.72%	2.72%	0.50%	4.11%
Interlab 1 Fb	4.26%	8.74%	3.64%	8.16%
Interlab 1 Ufa	1.64%	0.37%	2.31%	8.38%
Interlab 1 Ufb	4.55%	3.25%	0.75%	2.18%
Interlab 2 Fa	4.92%	4.39%	2.65%	0.75%
Interlab 2 Fb	1.65%	1.08%	0.11%	3.53%
Interlab 2 Ufa	0.00%	1.95%	7.85%	5.66%
Interlab 2 Ufb	0.90%	14.98%	1.26%	4.00%
Interlab 3 Fa	2.86%	0.29%	0.57%	1.44%
Interlab 3 Fb	3.51%	0.40%	2.92%	1.81%
Interlab 3 UFa	3.58%	4.97%	0.10%	14.36%
Interlab 3 Ufb	2.21%	1.17%	3.31%	0.59%

Field Replicates. Two replicate samples (a and b) were taken from each site, for both unfiltered and filtered samples. The results for total mercury were compiled for each replicate sample by averaging the analytical duplicate results to get the "duplicate average" (Figure 1). As described above, sometimes 2 analytical duplicates were run, and sometimes 3 for each replicate sample.

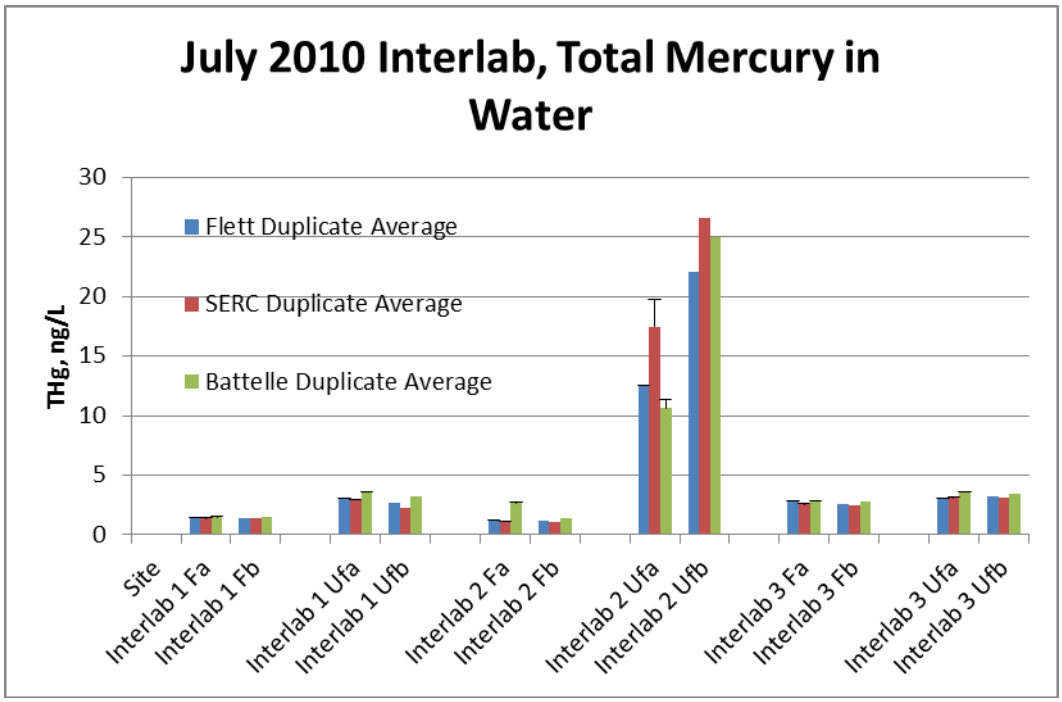


Figure 1. Total mercury concentrations measured by each laboratory in filtered and unfiltered water samples from the Penobscot River. Samples were collected July 28, 2010. "a" and "b" samples were two separate, replicate samples taken from the same site. Error bars are +/- 1 standard deviation. These bars were often too small to show up on the graph.

Because field replicates are two separate water samples, the RPD's between replicates is expected to be greater than the RPD's between analytical duplicates run on the same sample. This was the case (Table 2). The average RPD between replicates was 13.9 +/-21.0 % (Flett Research results), 13.0 +/- 16.6 % (SERC results) and 26.8 +/- 35.0 % (Battelle) . These RPD's are quite a bit higher than the duplicate RPD's, which averaged 2.6 %, 2.9 %, and 4.6 %, respectively. Interlab 2 Ufa and b replicate samples were especially different from each other (Figure 1, Table 2).

The RPD's between field replicates are not a measure of laboratory quality control, but are useful in establishing the expected variance in field samples from one site, and limitations in interpreting single samples from one site. The PRMS routinely takes two replicates for surface samples, and one sample for deeper depths where pumping time is an issue. The routine replicates are analyzed by one lab, and statistics on these are also useful, but the interlab samples presented give better statistical assurance on how much replicates are expected to vary, because each replicate was analyzed by three laboratories.

Table 2. Relative percent differences between field replicates taken at the same site.

THg, Field Replicate Sample	Flett Replicate RPD	SERC Replicate RPD	Battelle Relicate RPD
Interlab 1F a&b	1.1%	2.2%	0.7%
Interlab 1 UF a&b	14.6%	24.9%	10.7%
Interlab 2F a&b	0.8%	5.2%	61.1%
Interlab 2 UF a&b	55.5%	41.5%	80.9%
Interlab 3 F a&b	8.8%	3.8%	0.5%
Interlab 3 UF a&b	2.9%	0.5%	6.9%

Site Averages. Results for the two replicate samples were combined to get an average result from each laboratory for each sampling site (Figure 2).

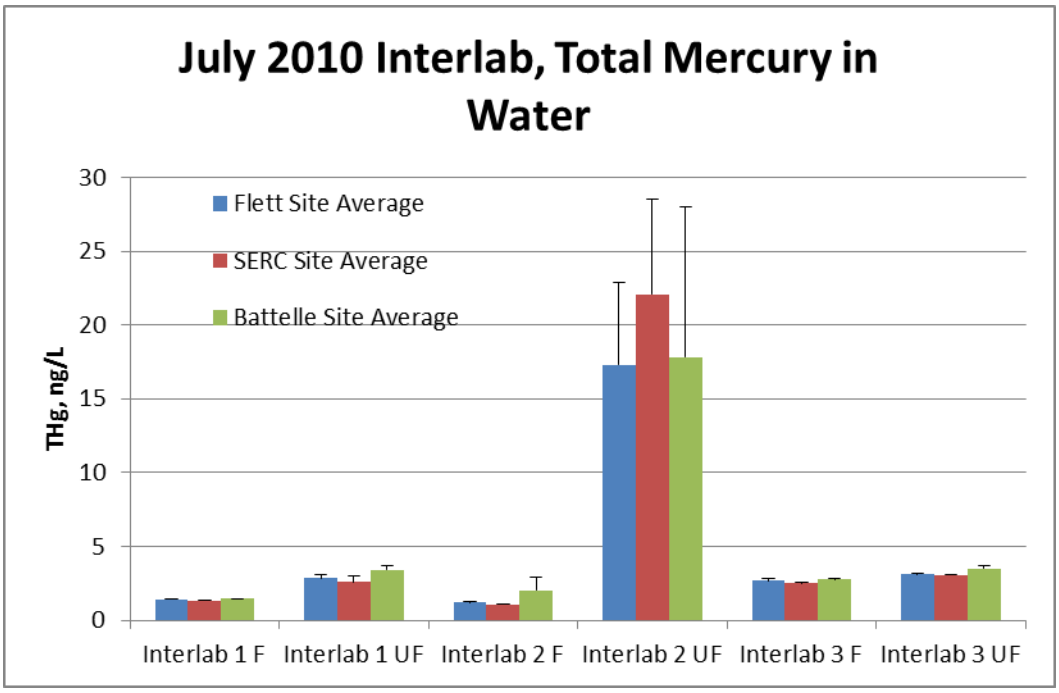


Figure 2. The average result for each site, from each laboratory. These averages were calculated from the two replicate samples taken at each site (a and b). The standard deviations were calculated from all results for that site from each lab.

Interlab Comparison for Total Mercury in Water. The standard approach to quantifying the reproducibility among the labs is first to calculate the grand average of all lab results for each site sampled. This is considered the "correct" analytical result for that site, because the actual value is unknown. Then each individual lab's result is compared to that average by calculating the RPD. These RPD's are shown in Table 3. All RPD's except for two were within the recommended guideline for replicate samples (<24% for total mercury in water, USEPA 2002).

Table 3. Relative percent difference between the result of each laboratory and the grand average result for each sample.

Site	Site Grand Average Total Hg, ng/L	Flett RPD, %	SERC RPD, %	Battelle RPD, %
Interlab 1 F	1.40	0.15%	4.24%	4.21%
Interlab 1 UF	2.94	3.31%	12.96%	14.33%
Interlab 2 F	1.44	17.26%	28.77%	34.06%
Interlab 2 UF	19.06	9.66%	14.64%	6.81%
Interlab 3 F	2.65	1.26%	6.12%	4.56%
Interlab 3 UF	3.23	3.38%	5.26%	8.11%

Methyl Mercury in Water

Analytical Duplicates

Each laboratory analyzed each sample for methyl mercury (MeHg) twice or three times. All of the RPD's between analytical duplicate results (Table 4) were within the EPA guideline of +/- 35% for MeHg in water (USEPA 1998). The average RPD for duplicates was 9 +/- 7%, for Flett Research, 6 +/- 5 % for Battelle, 11 +/- 9% for SERC, and 4 +/- 4% for Trent U.

Table 4. Relative percent differences between duplicate analyses for MeHg from one sample. "F" = filtered; "Uf"-= unfiltered.

Water Sample	Battelle Duplicate RPD	Flett Duplicate RPD	SERC Duplicate RPD	Trent Duplicate RPD
Interlab 1 Fa	2%	12%	17%, 4%	1%
Interlab 1 Fb	7%	20%, 12%	2%, 4%	4%
Interlab 1 Ufa	2%	15%	8%, 24%	10%
Interlab 1 Ufb	4%	7%, 24%	10%, 2%	3%
Interlab 2 Fa	17%	0%	24%, 24%	5%
Interlab 2 Fb	3%	0%	27%, 28%	12%
Interlab 2 Ufa	15%	12%	1%, 19%	8%
Interlab 2 Ufb	8%	9%, 9%	14%, 21%	4%
Interlab 3 Fa	7%	3%	2%, 5%	2%
Interlab 3 Fb	1%	0%, 8%	6%, 5%	1%
Interlab 3 UFa	1%	3%	3%, 5%	1%
Interlab 3 Ufb	6%	3%	6%, 14%	2%

Replicate Water Samples. At each site, two replicate samples (a and b) were taken for both filtered and unfiltered water (Figure 3).

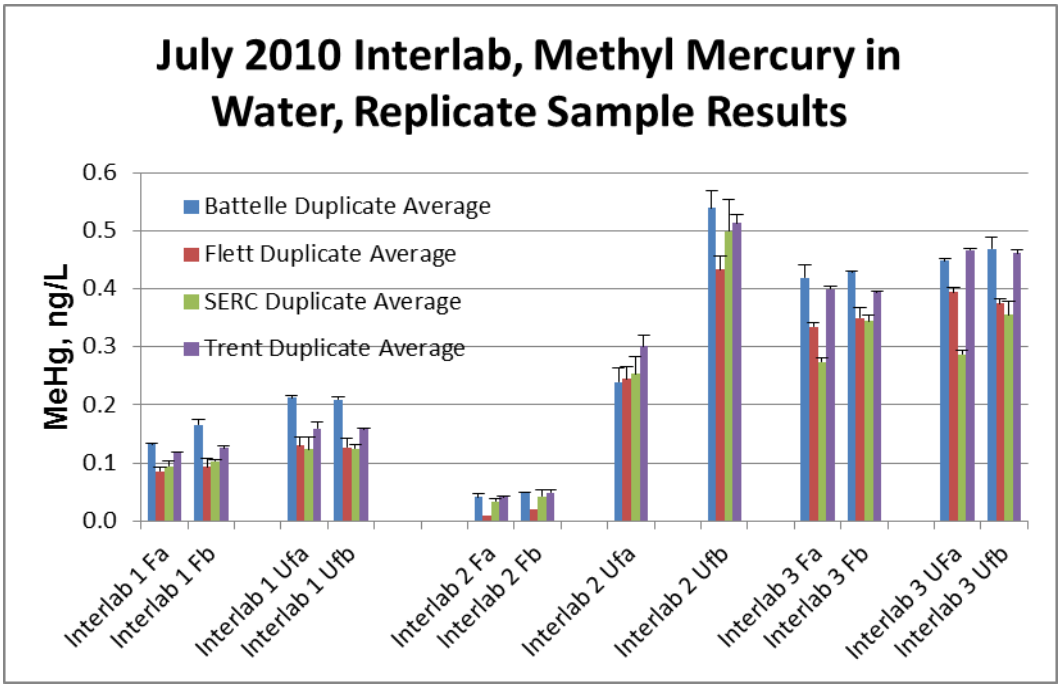


Figure 3. Methyl mercury concentrations measured by each laboratory in filtered and unfiltered water samples from the Penobscot River (July 28, 2010). Each replicate sample ("a" and "b") was analyzed twice or three times, and the average of these duplicate analyses is shown in the graph.

As expected, the RPD's between replicates (Table 5) were greater than analytical variability would explain. The average RPD between replicates was 21 +/- 29% (Battelle), 24 +/- 25% (Flett), 24 +/- 22 % (SERC) and 13 +/- 20% (Trent), compared to 9,6,11 and 4 % respectively for analytical duplicate RPD's (Table 4).

Table 5. Relative percent difference between results for replicate samples taken from the same site, analyzed for methyl mercury.

MeHg Field Replicate Sample	Battelle Replicate RPD, %	Flett Replicate RPD, %	SERC Replicate RPD, %	Trent Replicate RPD, %
Interlab 1 Fa&b	22.4%	9.3%	8.0%	6.1%
Interlab 1 Ufa&b	1.7%	2.6%	1.6%	0.9%
Interlab 2 Fa&b	17.1%	66.7%	23.1%	16.9%
Interlab 2 Ufa&b	77.0%	55.5%	65.3%	52.1%
Interlab 3 Fa&b	2.2%	4.4%	22.5%	1.5%
Interlab 3 Ufa&b	4.4%	5.2%	21.2%	1.3%

<u>Site Averages</u>. Results for the two replicate samples were combined to get an average results from each laboratory for each sampling site (Figure 4).

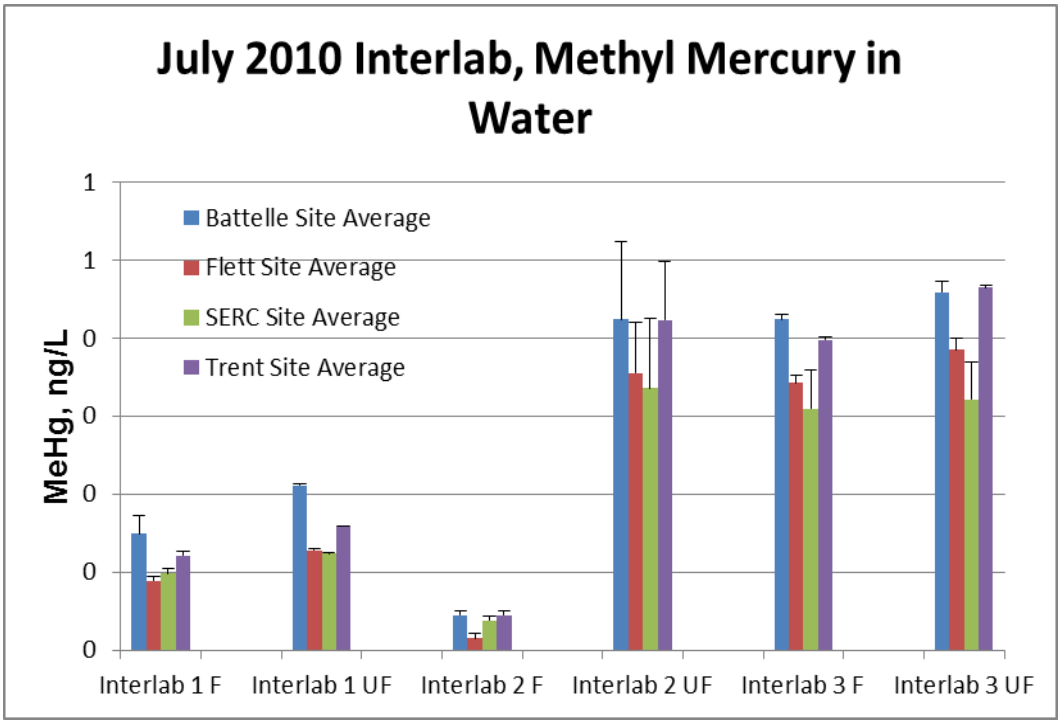


Figure 4. Average of samples A and B for each site. Error bars were calculated from all analytical duplicate results for each site, for each laboratory.

Interlab Comparison for Methyl Mercury in Water. The relative percent differences (RPD's) between the site average for methyl mercury from each laboratory, and the grand average (Table 6), were well within the recommended guideline for replicate samples of < 35% for methyl mercury in water. One result was very high in terms of RPD (Flett, Interlab 2 F), but this sample was extremely low in MeHg concentration, so even a small absolute difference leads to high relative difference. Thus, this one outlier is not considered a problem, and the overall results were very satisfactory.

Table 6. Relative percent differences (RPD's) for each lab compared to the 4 lab average, for methyl mercury in filtered water.

Site	Grand Average MeHg, ng/L	Battelle Interlab RPD %	Flett Interlab RPD %	SERC Interlab RPD %	Trent U. Interlab RPD %
Interlab 1 F	0.11	26%	25%	15%	6%
Interlab 1 UF	0.16	30%	19%	23%	2%
Interlab 2 F	0.04	23%	82%	6%	23%
Interlab 2 UF	0.38	10%	8%	14%	10%
Interlab 3 F	0.37	14%	7%	17%	8%
Interlab 3 UF	0.41	12%	6%	24%	13%

References.

U.S. Environmental Protection Agency Office of Water Office of Science and Technology Engineering and Analysis Division (4303), 1998. Method 1630: Methyl Mercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry.

United States Environmental Protection Agency, Office of Water 4303, 2002.
Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry. EPA-821-R-02-019

Appendix A. Total Mercury results from Flett Reserarch Ltd. July 2010 Total Mercury results, Penobscot Interlab Comparison.

				Flett Individual duplicate results	Flett Duplicate Average	Flett Site Average
Site	**Sample**	**Sample Details**	**Sample Type**	**Net Total Hg conc. (ng/L)**	**Net Total Hg conc. (ng/L)**	**Net Total Hg conc. (ng/L)**
Interlab 1 F	Interlab 1 Fa	Flett Inter1- Fa		1.4	1.40	1.40
		Flett Inter1- Fa	Repeat Aliquot	1.39		
	Interlab 1 Fb	Flett Inter1- Fb		1.38	1.41	
		Flett Inter1- Fb	Repeat Aliquot	1.44		
Interlab 1 UF	Interlab 1 Ufa	Flett Inter1- UFa		3.03	3.06	2.85
		Flett Inter1- UFa	Repeat Aliquot	3.08		
	Interlab 1 Ufb	Flett Inter1- UFb		2.58	2.64	
		Flett Inter1- UFb	Repeat Aliquot	2.7		
Interlab 2 F	Interlab 2 Fa	Flett Inter2- Fa		1.25	1.22	1.22
		Flett Inter2- Fa	Repeat Aliquot	1.19		
	Interlab 2 Fb	Flett Inter2- Fb		1.22	1.21	
		Flett Inter2- Fb	Repeat Aliquot	1.2		
Interlab 2 UF	Interlab 2 Ufa	Flett Inter2- UFa		12.5	12.50	17.30
		Flett Inter2- UFa	Repeat Aliquot	12.5		
	Interlab 2 Ufb	Flett Inter2- UFb		22	22.10	
		Flett Inter2- UFb	Repeat Aliquot	22.2		

Site	Sample	Sample Details	Sample Type	Net Total Hg conc. (ng/L)	Net Total Hg conc. (ng/L)	Net Total Hg conc. (ng/L)
Interlab 3 F	Interlab 3 Fa	Flett Inter3- Fa		2.84	2.80	2.68
		Flett Inter3- Fa	Repeat Aliquot	2.76		
	Interlab 3 Fb	Flett Inter3- Fb		2.61	2.57	
		Flett Inter3- Fb	Repeat Aliquot	2.52		
Interlab 3 UF	Interlab 3 UFa	Flett Inter3- UFa		3.02	3.08	3.12
		Flett Inter3- UFa	Repeat Aliquot	3.13		
	Interlab 3 Ufb	Flett Inter3- UFb		3.13	3.17	
		Flett Inter3- UFb	Repeat Aliquot	3.2		
		Q:\Clients M-Z\Penobscot\2010(214)\Total Mercury\TMWATR081610ZB1.xls				

Appendix B. Results for Total Mercury from Smithsonian Ecological Research Center. Penobscot Water Interlab July 2010.

			SERC Individual duplicate results	SERC Duplicate Average	SERC Site Average
Site	**SERC Sample ID**	**Method**	**THg (ng/L)**	**THg (ng/L)**	**THg (ng/L)**
Interlab 1 F	SERC Inter1-Fa #1	Filtered Pump	1.33	1.36	1.35
	SERC Inter1-Fa #2	Filtered Pump	1.37		
	SERC Inter1-Fa #3	Filtered Pump	1.38		
	SERC Inter1-Fb #1	Filtered Pump	1.27	1.33	
	SERC Inter1-Fb #2	Filtered Pump	1.39		
	SERC Inter1-Fb #3	Filtered Pump	1.34		
Interlab 1 UF	SERC Inter1-UFa #1	Unfiltered pump	2.94	2.91	2.58
	SERC Inter1-UFa #2	Unfiltered pump	2.93		
	SERC Inter1-UFa #3	Unfiltered pump	2.86		
	SERC Inter1-UFb #1	Unfiltered pump	2.32	2.26	
	SERC Inter1-UFb #2	Unfiltered pump	2.24		
	SERC Inter1-UFb #3	Unfiltered pump	2.23		
Interlab 2 F	SERC Inter2-Fa #1	Filtered Pump	1.15	1.11	1.08
	SERC Inter2-Fa #2	Filtered Pump	1.10		
	SERC Inter2-Fa #3	Filtered Pump	1.07		
	SERC Inter2-Fb #1	Filtered Pump	1.06	1.05	
	SERC Inter2-Fb #2	Filtered Pump	1.05		
	SERC Inter2-Fb #3	Filtered Pump	1.05		
Interlab 2 UF	SERC Inter2-UFa #1	Unfiltered pump	17.24	17.48	22.07
	SERC Inter2-UFa #2	Unfiltered pump	16.91		
	SERC Inter2-UFa #3	Unfiltered pump	18.29		
	SERC Inter2-UFb #1	Unfiltered pump	29.26	26.65	
	SERC Inter2-UFb #2	Unfiltered pump	25.18		
	SERC Inter2-UFb #3	Unfiltered pump	25.50		
Interlab 3 F	SERC Inter3-Fa #1	Filtered Pump	2.54	2.54	2.49
	SERC Inter3-Fa #2	Filtered Pump	2.53		
	SERC Inter3-Fa #3	Filtered Pump	2.55		
	SERC Inter3-Fb #1	Filtered Pump	2.46	2.44	

Site	SERC Sample ID	Method	THg (ng/L)	THg (ng/L)	THg (ng/L)
	SERC Inter3-Fb #2	Filtered Pump	2.47		
	SERC Inter3-Fb #3	Filtered Pump	2.40		
Interlab 3 UF	SERC Inter3-UFa #1	Unfiltered pump	2.95	3.06	3.06
	SERC Inter3-UFa #2	Unfiltered pump	3.10		
	SERC Inter3-UFa #3	Unfiltered pump	3.11		
	SERC Inter3-UFb #1	Unfiltered pump	3.01	3.07	
	SERC Inter3-UFb #2	Unfiltered pump	3.05		
	SERC Inter3-UFb #3	Unfiltered pump	3.15		

Appendix C. Total Mercury results from Battelle, July 2010 Water Interlab.

			Battelle Individual duplicate results	Battelle Duplicate Average	Battelle Site Average
Site	**Sample**	**Sample Type**	**THg (ng/L)**	**THg (ng/L)**	**THg (ng/L)**
Interlab 1 F	Battelle Inter1-Fa	Filtered water	1.49	1.46	1.47
	Battelle Inter1-Fa	Filtered water	1.43		
	Battelle Inter1-Fb	Filtered water	1.41	1.47	
	Battelle Inter1-Fb	Filtered water	1.53		
Interlab 1 UF	Battelle Inter1-Ufa	Unfiltered water	3.43	3.58	3.40
	Battelle Inter1-Ufa	Unfiltered water	3.73		
	Battelle Inter-1UFb	Unfiltered water	3.25	3.22	
	Battelle Inter-1UFb	Unfiltered water	3.18		
Interlab 2 F	Battelle Inter2-Fa	Filtered water	2.67	2.66	2.04
	Battelle Inter2-Fa	Filtered water	2.65		
	Battelle Inter2-Fb	Filtered water	1.44	1.42	
	Battelle Inter2-Fb	Filtered water	1.39		

Site	Sample	Sample Type	THg (ng/L)	THg (ng/L)	THg (ng/L)
Interlab 2 UF	Battelle Inter2-Ufa*	Unfiltered water	10.90	10.60	17.80
	Battelle Inter2-Ufa*	Unfiltered water	10.30		
	Battelle Inter2-Ufb*	Unfiltered water	25.50	25.00	
	Battelle Inter2-Ufb*	Unfiltered water	24.50		
Interlab 3 F	Battelle Inter3-Fa	Filtered water	2.80	2.78	2.77
	Battelle Inter3-Fa	Filtered water	2.76		
	Battelle Inter3-Fb	Filtered water	2.79	2.77	
	Battelle Inter3-Fb	Filtered water	2.74		
Interlab 3 UF	Battelle Inter3-Ufa	Unfiltered water	3.36	3.62	3.50
	Battelle Inter3-Ufa	Unfiltered water	3.88		
	Battelle Inter-1UFb	Unfiltered water	3.39	3.38	
	Battelle Inter-1UFb	Unfiltered water	3.37		

Appendix D. Methyl mercury results from Flett Research Ltd.

	Sampling Details	Net CH3Hg as Hg (ng/L) [recovery corrected]	Flett Duplicate Average	Flett Site Average
Site	**Site ID**	**MeHg, ng/L**	**MeHg, ng/L**	**MeHg, ng/L**
Interlab 1 F	Flett Inter1- Fa	0.09	0.09	0.09
	Flett Inter1- Fa	0.08		
	Flett Inter1- Fb	0.11	0.09	
	Flett Inter1- Fb	0.09		
	Flett Inter1- Fb	0.08		
Interlab 1 UF	Flett Inter1- UFa	0.14	0.13	0.13
	Flett Inter1- UFa	0.12		
	Flett Inter1- UFb	0.13	0.13	
	Flett Inter1- UFb	0.14		
	Flett Inter1- UFb	0.11		
Interlab 2 F	Flett Inter2- Fa	~0.01		
	Flett Inter2- Fa	~-0.01		
	Flett Inter2- Fb	~0.02		
	Flett Inter2- Fb	~0.02		
Interlab 2 UF	Flett Inter2- UFa	0.26	0.25	0.34
	Flett Inter2- UFa	0.23		
	Flett Inter2- UFb	0.42	0.43	
	Flett Inter2- UFb	0.46		
	Flett Inter2- UFb	0.42		
Interlab 3 F	Flett Inter3- Fa	0.33	0.34	0.34
	Flett Inter3- Fa	0.34		
	Flett Inter3- Fb	0.34	0.35	
	Flett Inter3- Fb	0.34		
	Flett Inter3- Fb	0.37		

Site	Site ID	MeHg, ng/L	MeHg, ng/L	MeHg, ng/L
Interlab 3 UF	Flett Inter3- UFa	0.4	0.40	0.39
	Flett Inter3- UFa	0.39		
	Flett Inter3- UFb	0.37	0.38	
	Flett Inter3- UFb	0.38		

Appendix E. Methyl Mercury results from SERC, July 2010 Water Interlab.

	SERC	**SERC Duplicate Average**	**SERC Site Average**
Site ID	**MeHg, ng/L**	**MeHg, ng/L**	**MeHg, ng/L**
SERC Inter1-Fa #1	0.10	0.09	0.10
SERC Inter1-Fa #2	0.09		
SERC Inter1-Fa #3	0.09		
SERC Inter1-Fb #1	0.10	0.10	
SERC Inter1-Fb #2	0.10		
SERC Inter1-Fb #3	0.11		
SERC Inter1-UFa #1	0.11	0.12	0.12
SERC Inter1-UFa #2	0.12		
SERC Inter1-UFa #3	0.15		
SERC Inter1-UFb #1	0.12	0.12	
SERC Inter1-UFb #2	0.13		
SERC Inter1-UFb #3	0.13		
SERC Inter2-Fa #1	0.04	0.03	0.04
SERC Inter2-Fa #2	0.03		
SERC Inter2-Fa #3	0.04		
SERC Inter2-Fb #1	0.05	0.04	
SERC Inter2-Fb #2	0.04		
SERC Inter2-Fb #3	0.03		
SERC Inter2-UFa #1	0.24	0.25	0.38
SERC Inter2-UFa #2	0.24		
SERC Inter2-UFa #3	0.29		
SERC Inter2-UFb #1	0.49	0.50	
SERC Inter2-UFb #2	0.56		
SERC Inter2-UFb #3	0.45		
SERC Inter3-Fa #1	0.27	0.27	0.31
SERC Inter3-Fa #2	0.27		
SERC Inter3-Fa #3	0.28		
SERC Inter3-Fb #1	0.35	0.34	
SERC Inter3-Fb #2	0.33		
SERC Inter3-Fb #3	0.35		

Site ID	**MeHg, ng/L**	**MeHg, ng/L**	**MeHg, ng/L**
SERC Inter3-UFa #1	0.28	0.29	0.32
SERC Inter3-UFa #2	0.29		
SERC Inter3-UFa #3	0.28		
SERC Inter3-UFb #1	0.36	0.36	
SERC Inter3-UFb #2	0.38		
SERC Inter3-UFb #3	0.33		

Appendix F. Methyl Mercury results from Battelle, July 2010 Penobscot Water Interlab.

-	-	**Battelle**	**Battelle Duplicate Average**	**Battelle Site Average**
Site	**Replicate Sample**	**MeHg, ng/L**	**MeHg, ng/L**	**MeHg, ng/L**
Interlab 1 F	Interlab 1 Fa	0.13	0.13	0.15
-	Interlab 1 Fa	0.13	-	-
-	Interlab 1 Fb	0.16	0.17	-
-	Interlab 1 Fb	0.17	-	-
-	-	-	-	-
Interlab 1 UF	Interlab 1 Ufa	0.21	0.21	0.21
-	Interlab 1 Ufa	0.22	-	-
-	Interlab 1 Ufb	0.21	0.21	-
-	Interlab 1 Ufb	0.21	-	-
-	-	-	-	-
Interlab 2 F	Interlab 2 Fa	0.04	0.04	0.05
-	Interlab 2 Fa	0.04	-	-
-	Interlab 2 Fb	0.05	0.05	-
-	Interlab 2 Fb	0.05	-	-
-	-	-	-	-
Interlab 2 UF	Interlab 2 Ufa	0.26	0.24	0.39
-	Interlab 2 Ufa	0.22	-	-
-	Interlab 2 Ufb	0.52	0.54	-
-	Interlab 2 Ufb	0.56	-	-
-	-	-	-	-
Interlab 3 F	Interlab 3 Fa	0.40	0.42	0.42
-	Interlab 3 Fa	0.44	-	-
-	Interlab 3 Fb	0.43	0.43	-
-	Interlab 3 Fb	0.43	-	-
-	-	-	-	-
Interlab 3 UF	Interlab 3 UFa	0.45	0.45	0.46
-	Interlab 3 UFa	0.45	-	-
-	Interlab 3 Ufb	0.45	0.47	-
-	Interlab 3 Ufb	0.48	-	-

Appendix G. Methyl mercury results from Trent U., July 2010 Penobscot Water Interlab.

-	-	**Trent**	**Trent Duplicate Average**	**Trent Site Average**
Site	**Sample ID**	**MeHg, ng/L**	**MeHg, ng/L**	**MeHg, ng/L**
Interlab 1 F	Trent Inter1-Fa	0.119	0.118	0.12
		0.117		
	Trent Inter1-Fb	0.127	0.125	
		0.123		
Interlab 1 UF	Trent Inter1 Ufa	0.168	0.159	0.16
		0.151		
	Trent Inter1 Ufb	0.160	0.158	
		0.156		
Interlab 2 F	Trent Inter2-Fa	0.040	0.041	0.05
		0.042		
	Trent Inter2-Fb	0.046	0.049	
		0.052		
Interlab 2 UF	Trent Inter2-Ufa	0.289	0.302	0.41
		0.314		
	Trent Inter2-Ufb	0.505	0.514	
		0.524		
Interlab 3 F	Trent Inter3-Fa	0.397	0.400	0.40
		0.404		
	Trent Inter3-Fb	0.395	0.394	
		0.393		
Interlab 3 UF	Trent Inter3-Ufa	0.470	0.467	0.46
		0.465		
	Trent Inter3-Ufb	0.466	0.461	
		0.456		

Report on Radioisotope Inter-lab Comparisons

Penobscot River Mercury Project

October 2012

C.A. Kelly

R&K Research Inc.

The three laboratories that have analyzed sediment core samples for radioisotopes, for the Penobscot River Mercury Project, are:

1. Texas A&M University at Galveston (TAMUG), under direction of Dr. Peter Santschi.
2. Southern Mississippi University (SMU), under direction of Dr. Kevin Yeager
3. Flett Research Ltd, under direction of Dr. Robert Flett.

All three of these laboratories have extensive experience in making the measurements required for the PRMS, which are Pb-210, Cs-137, Be-5, and Ra-226. Cores were taken and sectioned in the field by Dr. Yeager. Core samples were analyzed first for mercury at Flett Research Ltd to see if the Hg profile data indicated that core was taken from an interpretable (physically undisturbed) site. If so, then radioisotope activities were measured. Approximately 1/3 of the cores were done at Flett Research, 1/3 at TAMUG, and 1/3 at SMU. Interpretation of profiles was done by Dr. Santschi.

Because radioisotopes were measured in three laboratories, inter lab comparison of the radioisotope results was a necessary part of data verification for these cores. This report focuses on the inter lab comparisons of results for Pb-210 and Cs-137, carried out in November 2010 and February 2011. All laboratories reported dpm/gdw (disintegrations per minute per gram dry weight of sediment), with Pb-210 done by alpha counting, and Cs-137 by gamma counting. Methods, standardization and corrections for counting efficiencies are available from the individual laboratories.

In order to compare the results, two types of statistics were calculated. One was to obtain the %RPD (Relative Percent Difference) between duplicate counts made on the same sample, in each laboratory. The rate of decay is accurately known for any radioisotope, and a measurement of decay activity would reflect this rate absolutely if measured over a long enough time period. However, this is usually not practical, and so variability in counts due to counting over time periods that are shorter than ideal is expected. Calculation of the RPD between duplicate counts is as follows:

RPD (%) = [(Absolute value of Count 1- Count 2)/(Average of Count 1 and Count 2)] * 100

This RPD is the minimum range of differences that are expected from the counting methods used, when comparing one lab to another.

The second type of statistic was to gather results for the same core sample from all three laboratories. These were evaluated by first calculating the average result of the three labs to obtain the “grand mean”, and then calculating the “inter-laboratory RPD”, which is the RPD between each lab’s result and this average result. These inter-laboratory RPD’s are expected to be larger than the RPD’s for duplicate counts of the same sample within one laboratory.

Pb-210.

Reproducibility in duplicate counts for the same sample. In some, but not all cases, samples included in the inter lab comparison exercises were counted twice. The results of these duplicate counts are in Table 1.

Table 1. RPD's for duplicate alpha counts of total Pb-210, for the same sample counted twice. Duplicate counts were not reported for all samples.

Core Section	Inter lab Date	Laboratory	Average Pb-210, dpm/g	Pb-210, RPD for duplicates
MM-3-B-09V, 4-5 cm	November 2010	Flett	6.10	5.31%
MM-3-B-09V, 85-90 cm	November 2010	Flett	2.85	2.44%
MM-3-B-09V, 85-90 cm	November 2010	USM	2.85	15.56%
MM 6A-9-10 cm	February 2011	Flett	6.14	0.33%
MM 6A-65-70 cm	February 2011	Flett	0.65	0.64%
PBR 29A-9-10 cm	February 2011	Flett	2.45	11.36%
PBR 29A-65-70 cm	February 2011	Flett	0.66	14.99%

Almost all of the RPD's for these duplicate counts were $\leq$ 15%.

Additional duplicate counts for Pb-210 were done for a representative sampling of core sections analyzed for the PRMS (Appendix A). A graph of these results showed that RPD's were related to total counts for the sample, i.e., for samples with counts greater than 2 dpm/g, RPD's were usually $\leq$ 15%. For samples with counts between 1 and 2 dpm/g, RPD's ranged from 3 to 27%, and for counts less than 1 dpm/g, RPD's were as high as 50%.

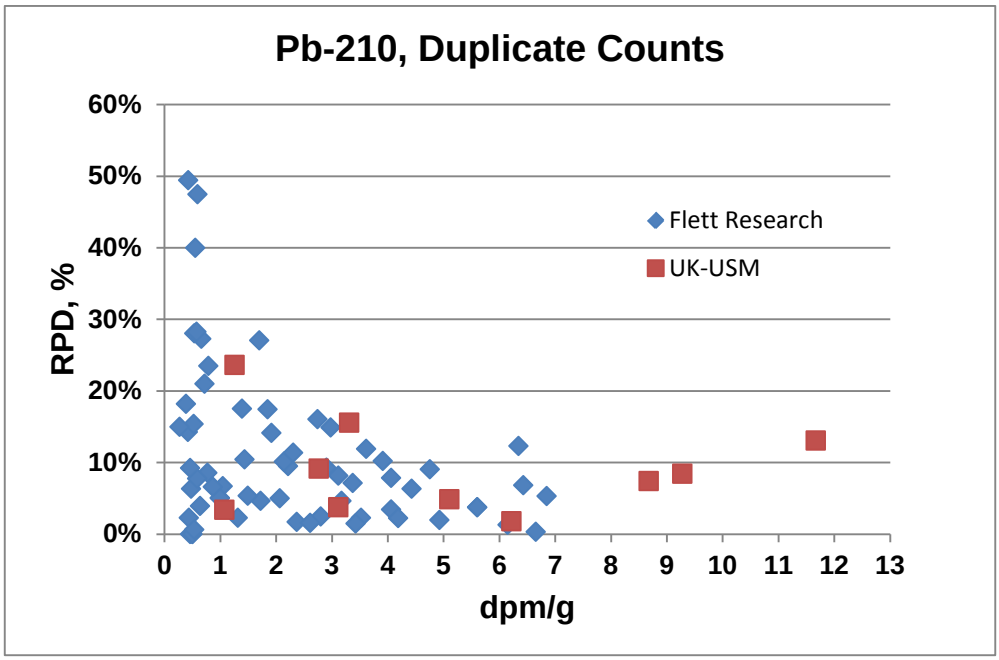


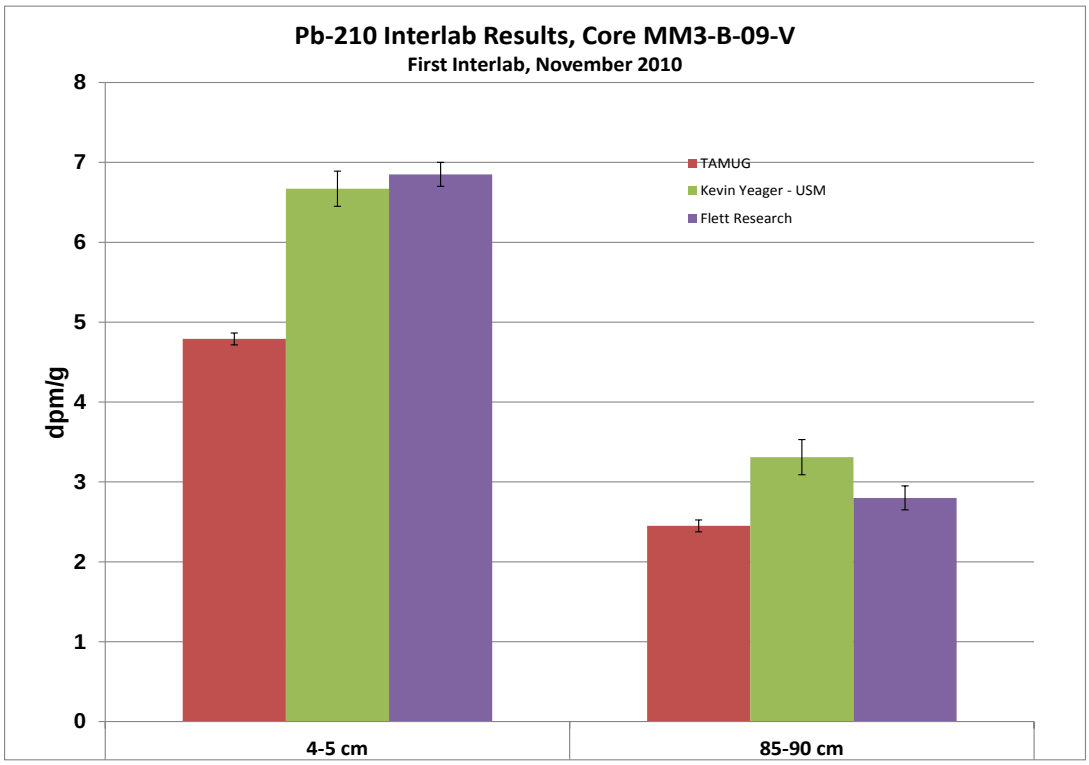
Figure 1. Relative per cent difference between duplicate counts of the same sample.

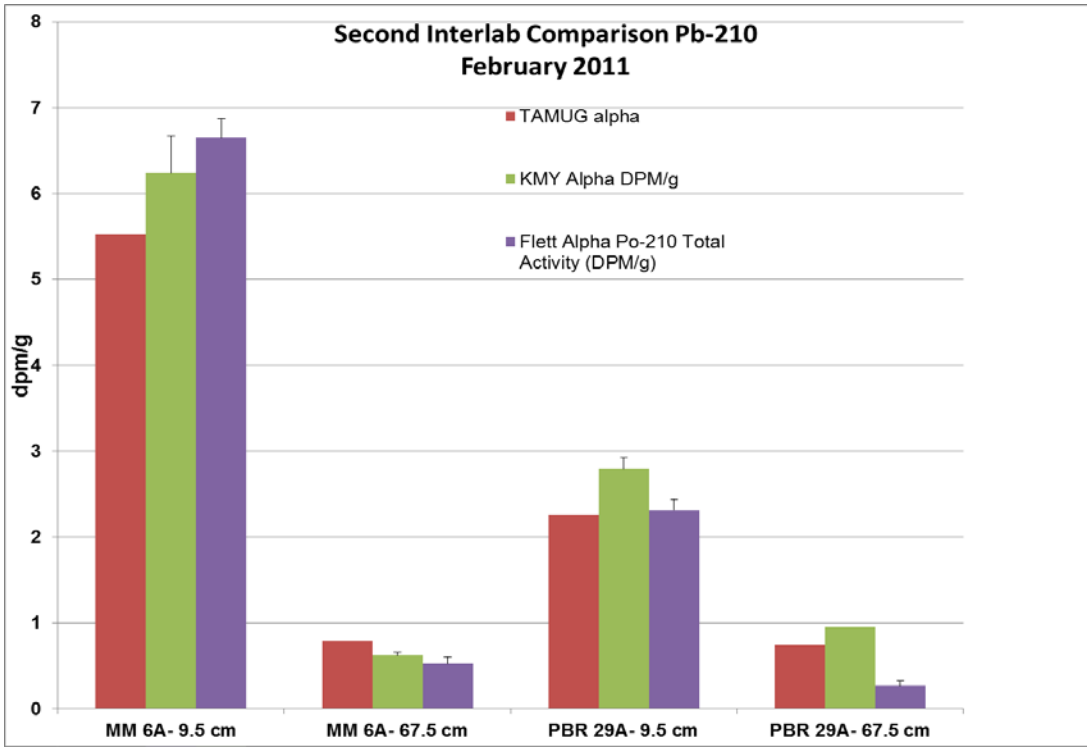
Inter lab Comparisons—Pb-210. The first inter lab comparison was done in November, 2010 (Core MM3-B-09V), and the second in February 2011 (Cores MM-06A-09V and PBR-29A-09V). Two sections were used from each core, for a total of 6 samples.

All of the results for a single core section were averaged to obtain the mean result for all 3 laboratories. This is called the "grand mean" and is taken to be the best estimate of the actual concentrations of Pb-210. Then the %RPD between each lab's result and this grand mean is calculated.

Based on the duplicate count results, the acceptable magnitudes of the inter-laboratory comparison RPD's are dependent on the absolute values for dpm/gdw. In samples with very low counts, a small absolute difference can translate into a large relative percent difference. Further, it would not be expected that RPD's for the result of each laboratory, compared to the grand mean, would be less than the RPD's for the same sample counted twice. Thus, for samples with absolute counts > 2 dpm/g, the acceptable difference among laboratories is equal to or slightly greater than 15%. For samples with counts between 1 and 2 dpm/g, acceptable RPD's would be equal to or slightly greater than 27%, and for counts less than 1 dpm/g, RPD's equal to or slightly greater than 50%.

In the first inter lab comparison exercise, two sections from Core MM3-B-09-V were measured for Pb-210, via alpha counting of Po-210 (Figure 1). In the second inter lab exercise, two sections from each of two additional cores (MM 6A and PBR 29A) were analyzed (Figure 2).





Figures 2 and 3. Pb-210 results from the three laboratories.

In order to compare the results from the different laboratories the RPD's between each laboratory's result and the grand mean of all 3 laboratories was calculated (Tables 2 and 3).

The criteria that were based on results of duplicate counts of the same sample, were met in all cases of comparison among the laboratories. For samples where average counts were greater than 2 dpm/g, the expectation was that RPD's would be equal to or slightly greater than 15%. . All results except for two were < 15%, and these two were only a small amount above this percentage—16% and 21% (Table 2). This is likely acceptable, since the variability among labs would be expected to increase the RPD somewhat over what was achieved for the same sample being counted twice in one laboratory. For samples with average counts less than 2 dpm/g, much higher variability is expected, and this was the case for 2 results where average counts were 0.66 dpm/g, and RPD's were 45% and 59% (Table 3). Again, this is consistent with the variability seen for duplicate counts in samples with low values (Figure 1).

Table 2. %RPD between each lab and the grand mean of all labs, Pb-210, Core MM-3-B-09V, December 2010.

	MM-3-B-09V, 4-5 cm, Average dpm/g = 6.10	**MM-3-B-09V, 85-90 cm, Average dpm/g = 2.85**
TAMUG	21.52%	14.14%
USM	9.28%	16.00%
Flett	12.23%	1.87%

Table 3. %RPD between each lab and the grand mean of all labs, Pb-210, Cores MM 6A and PBR 29A, February 2011.

	MM 6A, 9-10 cm, Average dpm/g = 6.14	**MM6A, 65-70 cm, Average dpm/g = 0.65**	**PBR 29A, 9-10 cm, Average dpm/g = 2.45**	**PBR 29A, 65-70 cm, Average dpm/g = 0.66**
TAMUG	10.0%	22.3%	7.9%	13.7%
USM	1.7%	4.2%	13.8%	45.1%
Flett	8.3%	18.1%	5.9%	58.8%

Cs-137

Duplicate counts of the same sample were provided for the Inter lab samples (Table 4), and for a representative number of other sections analyzed for the PRMS (Figure 4, Appendix B).

Table 4. RPD's for duplicate gamma counts of Cs-137, for the same sample counted twice. Core MM-3-B-09V, analyzed in November 2010. Duplicate counts were not reported for all samples.

Core Section	Inter lab Date	Laboratory	Average Cs-137, dpm/g	RPD for duplicates
MM-3-B-09V, 4-5 cm	Nov 2010	Flett	0.67	0.88%
MM-3-B-09V, 85-90 cm	Nov 2010	Flett	0.97	9.88%
MM 6A-9-10 cm	Feb 2011	Flett	0.81	168.00%, 20.80%
MM 6A-9-10 cm	Feb 2011	TAMUG	0.81	26.25%
MM 6A-65-70 cm	Feb 2011	Flett	0.02	345.45%
MM 6A-65-70 cm	Feb 2011	TAMUG	0.02	18.58%
PBR 29A-9-10 cm	Feb 2011	Flett	0.61	2.11%
PBR 29A-9-10 cm	Feb 2011	TAMUG	0.61	21.76%
PBR 29A-65-70 cm	Feb 2011	Flett	-0.01	53.33%
PBR 29A-65-70 cm	Feb 2011	TAMUG	-0.01	184.32%

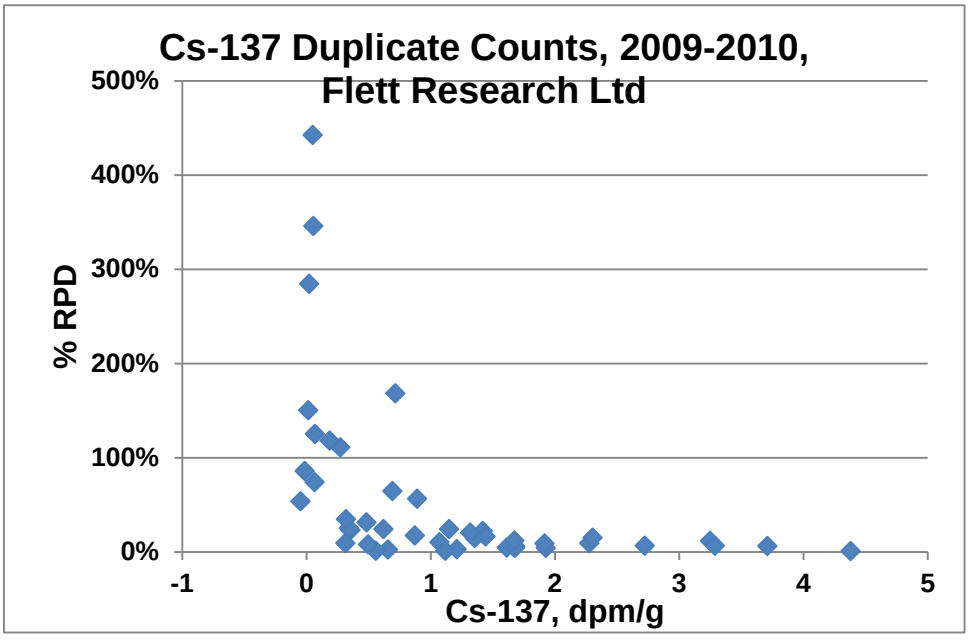
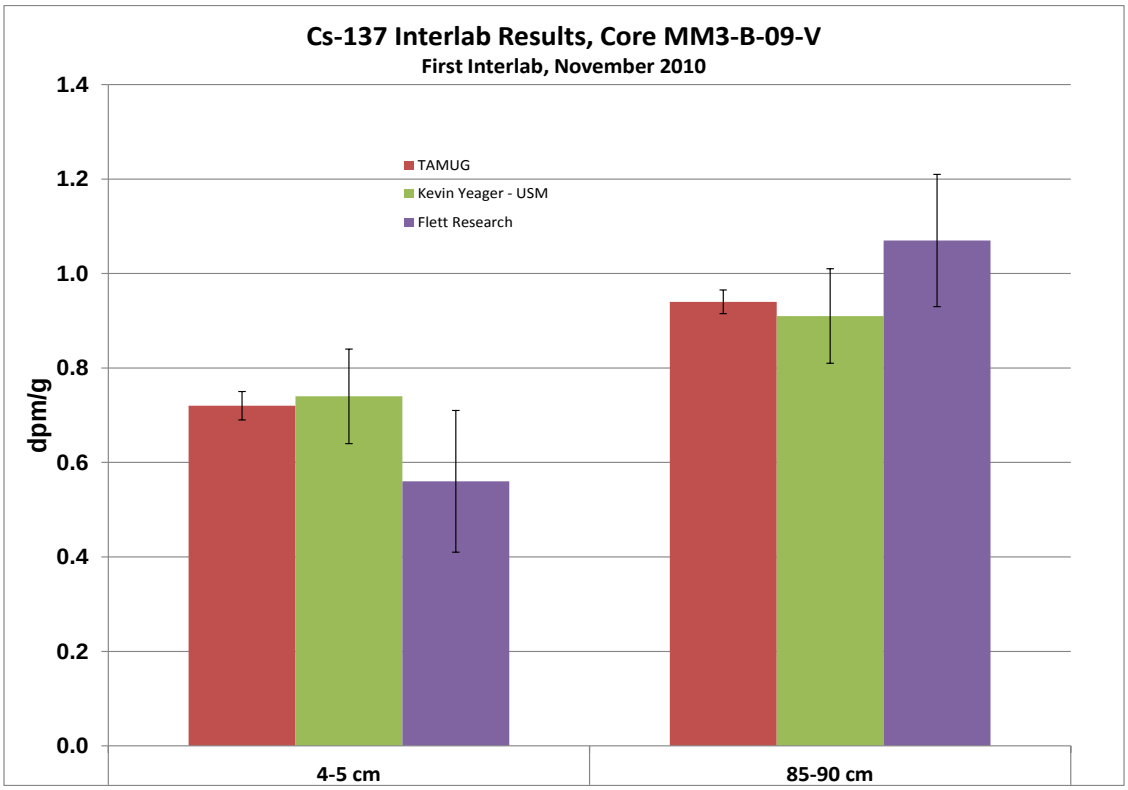
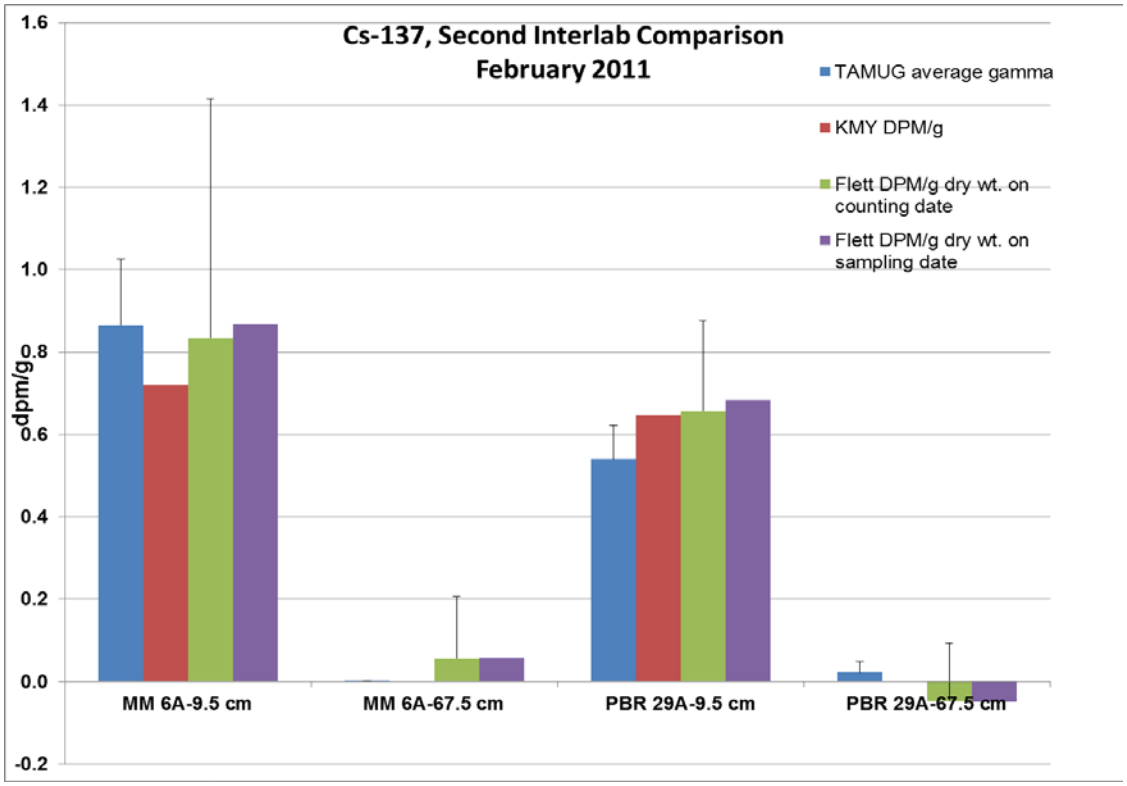


Figure 4. Relative per cent differences for duplicate counts for Cs-137 of the same core section.

The duplicate count data show that RPD's can often be higher than 25%, and up to 450%, for counts of < 1 dpm/g (Figure 4).

Absolute values of counts for all sections used in the inter laboratory comparison were <1 dpm/g, and counts for the deeper sections were <0.1 dpm/g. With such high relative differences seen between duplicate counts in this range, it is hard to evaluate whether there were real differences among the laboratories. A simple visual comparison of results from the three different laboratories showed quite good agreement (Figures 5 and 6). The three laboratories consistently distinguished between samples with counts in the higher range of 0.5 to 0.8 dpm/g from samples in the lower range of <0.1 dpm/g (Figure 6). The statistical calculations comparing each laboratory's result with the grand mean showed high RPD's (Tables 5 and 6), which was the same as the high RPD's seen for duplicate counts of a single sample (Table 4). Again, a small absolute difference between two counts that are very low can translate into a large relative difference, when the average absolute count is very low. Thus, in the case of comparing Cs-137 counts for these sections, the similar magnitudes of the absolute counts is a better measure of satisfactory agreement among the laboratories (Figures 5 and 6).





Figures 5 and 6. Cs-137 results from the three laboratories.

Table 5. Relative percent difference (RPD) between each lab and the grand mean of all labs, Cs-137, November 2010.

	MM-3-B-09V, 4-5 cm	**MM-3-B-09V, 85-90 cm**
TAMUG	6.93%	3.42%
USM	9.90%	6.51%
Flett Research	16.83%	9.93%

Table 6. Relative percent difference (RPD) between each lab and the grand mean of all labs, Cs-137, February 2011.

	MM 6A-9.5 cm	**MM 6A-67.5 cm**	**PBR 29A-9.5 cm**	**PBR 29A-67.5 cm**
TAMUG	7.2%	88.8%	12.2%	361.5%
USM	10.6%	100.0%	5.4%	100.0%
Flett Research	3.4%	188.8%	6.8%	461.5%

Appendix A. Duplicate counts for Pb-210.in core sections for PRMS.

Date/File	Result 1	Result 2	Average	RPD
	dpm/g	dpm/g	dpm/g	%
Flett Research Ltd.				
Nov 2006 to Jan 2011				
PBR-14R-C-09V(18-19 cm)	4.29	4.57	4.43	6.32%
PBR-14R-C-09V(22-24 cm)	2.32	2.11	2.22	9.48%
MM-5-C-O9V (9-10 cm)	5.71	5.5	5.61	3.75%
MM-5-C-O9V (30-32 cm)	1.02	0.97	1.00	5.03%
MM-5-C-O9V (50-55 cm)	0.5	0.5	0.50	0.00%
MM-12-C-09V (9-10 cm)	0.46	0.61	0.54	28.04%
MM-12-C-09V (19-20 cm)	0.46	0.49	0.48	6.32%
MM-12-C-09V (45-50 cm)	0.57	0.75	0.66	27.27%
OR-3-A-09V (9-10 cm)	3.56	3.48	3.52	2.27%
OR-3-A-09V (28-30 cm)	1.93	1.47	1.70	27.06%
OR-3-A-09V (65-70 cm)	0.64	0.79	0.72	20.98%
PBR-16-A-09V (7-8 cm)	3.4	3.45	3.43	1.46%
PBR-16-A-09V (16-17 cm)	2.35	2.39	2.37	1.69%
PBR-31-C-09V (9-10 cm)	4.97	4.54	4.76	9.04%
PBR-31-C-09V (20-22 cm)	1.76	1.68	1.72	4.65%
PBR-31-C-09V (65-70 cm)	0.32	0.53	0.43	49.41%
ES-2-C-09V (18-19 cm)	3.49	3.25	3.37	7.12%
ES-2-C-09V (75-80 cm)	0.44	0.66	0.55	40.00%
ES-3-C-09V (15-16 cm)	1.51	1.36	1.44	10.45%
ES-3-C-09V (19-20 cm)	0.45	0.73	0.59	47.46%
ES-3-C-09V (65-70 cm)	0.42	0.35	0.39	18.18%
ES-4-C-09V (5 cm)	3.83	3.4	3.62	11.89%
ES-4-C-09V (11 cm)	2.96	2.52	2.74	16.06%
ES-4-C-09V (34 cm)	0.46	0.46	0.46	0.00%
MM-2-B-09V (9-10 cm)	6.11	6.19	6.15	1.30%
MM-2-B-09V (19-20 cm)	4.13	3.99	4.06	3.45%
MM-2-B-09V (38-40 cm)	2.77	3.04	2.91	9.29%
MM-2-B-09V (85-90 cm)	1.53	1.45	1.49	5.37%
MM-2-B-09V (125-130 cm)	1.08	1.01	1.05	6.70%
MM-5-C-O9V (9-10 cm)	5.71	5.5	5.61	3.75%
MM-5-C-O9V (30-32 cm)	1.02	0.97	1.00	5.03%
MM-5-C-O9V (50-55 cm)	0.5	0.5	0.50	0.00%
MM-9-B-09V (9-10 CM)	4.11	3.71	3.91	10.23%
MM-9-B-09V (28-30 CM)	1.33	1.3	1.32	2.28%
MM-9-B-09V (60-65 CM)	0.45	0.39	0.42	14.29%
PBR-27-B-09V (32-34 cm)	0.43	0.44	0.44	2.30%
PBR-27-B-09V (45-50 cm)	0.48	0.56	0.52	15.38%

Date/File	Result 1	Result 2	Average	RPD
	dpm/g	dpm/g	dpm/g	%
OR-5-C-09V (10-11 cm)	4.972561	4.874823	4.92	1.99%
OR-5-C-09V (38-40 cm)	1.267555	1.510817	1.39	17.51%
OR-5-C-09V (55-60 cm)	0.653054	0.491436	0.57	28.24%
MM-3-B-09V (4-5 cm)	7.027379	6.663633	6.85	5.31%
MM-3-B-09V (85-90 cm)	2.835303	2.766881	2.80	2.44%
ES-7-A-09V (14-15 cm)	2.24601	2.029138	2.14	10.15%
ES-7-A-09V (38-40 cm)	0.838769	0.895969	0.87	6.59%
ES-7-A-09V (85-90 cm)	0.805018	0.738972	0.77	8.56%
ES-14-C-09V (0-1 cm)	4.235861	4.142932	4.19	2.22%
ES-14-C-09V (17-18 cm)	3.248267	3.10034	3.17	4.66%
ES-14-C-09V (50-55 cm)	2.985558	3.23933	3.11	8.15%
MM-6-A-09V (10cm)	6.66302	6.640738	6.65	0.33%
MM-6-A-09V (70 cm)	0.536658	0.533221	0.53	0.64%
PBR-29-A-09V (10 cm)	2.437342	2.175446	2.31	11.36%
PBR-29-A-09V (70 cm)	0.290487	0.249971	0.27	14.99%
PBR-6-C-09V (4-5 cm)	2.007976	1.686327	1.85	17.41%
PBR-6-C-09V (28-30 cm)	0.607965	0.562514	0.59	7.77%
PBR-6-C-09V (70-75 cm)	0.647783	0.622852	0.64	3.92%
PBR-10-A-O9V (18-19 cm)	6.733311	5.953255	6.34	12.30%
PBR-10-A-O9V (60-65 cm)	0.437229	0.479563	0.46	9.24%
PBR-10-A-O9V (10-11 cm)	6.650252	6.210625	6.43	6.84%
PBR-10-A-O9V (40-45 cm)	0.87742	0.692887	0.79	23.50%
PBR-11-B-09V (19-20 cm)	2.013738	2.117127	2.07	5.01%
PBR-11-B-09V (28-30 cm)	1.779659	2.050375	1.92	14.14%
PBR-19-A-O9V (5-6 cm)	3.902016	4.220971	4.06	7.85%
PBR-19-A-O9V (20-22 cm)	3.197017	2.753899	2.98	14.89%
PBR-19-A-O9V (45-50 cm)	2.630118	2.589065	2.61	1.57%
Yeager- USM-UK				
MM_03B_09V (0-1 cm)	8.354173	8.996802	8.68	7.41%
MM_03B_09V (17-18 cm)	4.979269	5.228479	5.10	4.88%
MM_03B_09V (20-22 cm)	6.160773	6.272536	6.22	1.80%
MM_03B_09V (85-90 cm)	3.052982	3.567979	3.31	15.56%
MM_04C_09V (0-1 cm)	8.884811	9.668744	9.28	8.45%
MM_04C_09V (20-22 cm)	3.055436	3.172831	3.11	3.77%
MM_04C_09V (85-90 cm)	1.108414	1.405521	1.26	23.64%
MM_06A_09V (0-1 cm)	12.43352	10.90775	11.67	13.07%
MM_06A_09V (20-22 cm)	2.892298	2.639942	2.77	9.12%
MM_06A_09V (85-90 cm)	1.055414	1.092077	1.07	3.41%

Appendix B. Duplicate Counts for Cs-137 in PRMS core sections.

Sample ID	%RPD between duplicate counts	Average Count	Result 1	Result 2
PBR-2-B-09V (125-130 cm)	0.57%	4.38	4.39	4.37
PBR-11-B-09V (34-36 cm)	5.90%	3.71	3.82	3.60
MM-5-C-O9V (18-19 cm)	11.38%	3.25	3.43	3.06
PBR-2-B-09V (80-85 CM)	6.12%	3.29	3.19	3.39
PBR-2-B-09V (85-90 CM)	6.45%	2.72	2.81	2.64
MM-5-C-O9V (19-20 cm)	9.08%	2.28	2.17	2.38
PBR-16-A-09V (18-19 cm)	14.77%	2.30	2.13	2.47
MM-9-B-09V (15-16 cm)	3.80%	1.93	1.89	1.96
MM-5-C-O9V (17-18 cm)	8.54%	1.92	1.84	2.00
MM-9-B-09V (14-15 cm)	4.36%	1.61	1.65	1.58
PBR-14R-C-09V (38-40 cm)	3.93%	1.68	1.64	1.71
MM-2-B-09V (55-60 cm)	5.56%	1.68	1.63	1.73
MM-2-B-09V (60-65 cm)	11.55%	1.67	1.58	1.77
PBR-19-A-O9V (14-15 cm)	21.98%	1.42	1.57	1.26
PBR-2-B-09V (17-18 CM)	16.24%	1.44	1.56	1.33
PBR-6-C-09V (6-7 cm)	24.06%	1.15	1.29	1.01
PBR-31-C-09V (26-28 cm)	14.44%	1.35	1.26	1.45
28 cm (26-28 cm)	2.68%	1.21	1.23	1.19
MM-9-B-09V (22-24 CM)	20.21%	1.32	1.19	1.45
PBR-10-A-O9V (6-7 cm)	56.24%	0.89	1.14	0.64
MM-3-B-09V 85-90 cm	9.88%	1.07	1.12	1.02
PBR-10-A-O9V (28-30 cm)	1.01%	1.12	1.12	1.11
OR-3-A-09V (26-28 cm)	17.05%	0.87	0.95	0.80
ES-4-C-09V (7 cm)	64.21%	0.69	0.92	0.47
PBR-29-A-09V 9-10 cm	2.11%	0.66	0.65	0.66
MM-3-B-09V 4-5 cm	0.88%	0.56	0.56	0.55
MM-2-B-09V (19-20 cm)	24.01%	0.62	0.55	0.69
MM-2-B-09V (4-5 cm)	7.69%	0.50	0.48	0.52
PBR-27-B-09V (45-50 cm)	110.69%	0.27	0.42	0.12
ES-14-C-09V (14-15 cm)	31.17%	0.48	0.41	0.56
ES-3-C-09V (8-9 cm)	22.87%	0.35	0.39	0.31
ES-14-C-09V (12-13 cm)	25.00%	0.34	0.38	0.30
OR-3-A-09V (34-36 cm)	34.43%	0.32	0.37	0.26
ES-7-A-09V (28-30 cm)	8.87%	0.31	0.33	0.30
ES-2-C-09V (38-40 cm)	117.65%	0.19	0.30	0.08
ES-7-A-09V (18-19 cm)	442.11%	0.05	0.16	-0.06

Sample ID	%RPD between duplicate counts	Average Count	Result 1	Result 2
MM-6-A-09V 65-70 cm	345.45%	0.06	0.15	-0.04
MM-6-A-09V 9-10 cm	168.00%	0.72	0.11	1.32
ES-7-A-09V (70-75 cm)	125.00%	0.07	0.11	0.03
MM-12-C-09V (22-24 cm)	73.73%	0.07	0.04	0.09
MM-12-C-09V (12-13 cm)	150.00%	0.01	0.00	0.03
MM-12-C-09V (13-14 cm)	85.71%	-0.01	-0.01	-0.02
ES-7-A-09V (20-22 cm)	284.21%	0.02	-0.01	0.05
PBR-29-A-09V 65-70 cm	53.33%	-0.05	-0.06	-0.03